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Final report

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Contents

1 Acknowledgments

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Abbreviations

ACIAR	Australian Centre for International Agricultural Research
ARF	Agrarian Research Foundation
aSSD	accelerated Single Seed Descent

BARI	Bangladesh Agricultural Research Institute
BAU	Bangladesh Agricultural University
BCR	Benefit/cost ratio
BWMRI	Bangladesh Wheat and Maize Research Institute
CRISPR	Clustered regularly interspaced short palindromic repeats
CSIRO	Commonwealth Scientific and Industrial Research Organization
DAE	Department of Agricultural Extension
FGD	Focus group discussion
GFP	Green fluorescent protein
GWAS	Genome-wide association study
Hyg	Hygromycin
JAF	John Allwright Fellow
HH	Household
KGF	Krishi Gobeshona Foundation
ICARDA	International Center for Agricultural Research in the Dry Areas
IITA	International Institute for Tropical Agriculture
MTR	Mid-Term Review
OFRD	On-Farm Research Division, BARI
PEP	Poverty Eradication Program
PRC	Pulse Research Centre, BARI
RIL	Recombinant inbred line
SOP	Standard operating practice
Tk	Bangladeshi taka
UWA	University of Western Australia
WL	Waterlogging
Yr1, Yr2	2017/18, 2019
Yr3, Yr4	2020, 2021/22
$	US $

2 Executive summary

The Government of Bangladesh (GoB) prioritized the coastal zone as that in most need for development. It is home to a population of ~40 M of whom 65% live below poverty line - compared with 24% for the whole country. Coastal Bangladesh has large areas of flood-prone land with variable levels of salinity and low agricultural production. Agriculture in the region centres around the annual cropping of monsoonal rice. Cropping in the dry rabi season is profitable, but limited by land topography/ drainage, soil salinity and irrigation availability. Opportunities exist for more profitable dry season cropping by exploiting significant areas of fallow land between rice crops. Within this overall landscape the flooding risk is determined largely by micro-topography, and salinity varies spatially and temporally determining potential land use. The overarching research question of the project is - How can we increase small-holder household incomes through improved productivity and profitability of dry-season crops on non-saline land, and with pulses and wheat with improved salinity tolerance on saline land in Southern Bangladesh?

The project started in June 2017 with ACIAR support (AUD$ 2.0 m) to Mar. 2023, supplemented in November 2017 with three years of co-funding from the Krishi Gobeshona Foundation (KGF) (A$ 0.48 m) for on-farm research. The project was led by the University of Western Australia (UWA) and CSIRO was the key Australian partner on wheat salinity research. In-country, the main agronomic research partners were the Bangladesh Agricultural Research Institute (BARI) and the Bangladesh Wheat and Maize Research Institute (BWMRI) - the key wheat research partner institution. The socio-economic component was undertaken by Bangladesh Agriculture University (BAU) and the NGOs - Agrarian Research Foundation (ARF) and the Poverty Eradication Program (PEP).

Component 1 of the project was to understand the context for practice change in dry season cropping in Southern Bangladesh and establish its evolution as a response to the project. A survey conducted using an independent sample was conducted in 2021 by BAU to understand the context for practice change in dry season cropping in non-saline areas, and to compare practices between focal farmers and non-focal farmers in neighbouring villages in 240 farmer households (HH), comprising 120 focal (hosted demonstrations) and 120 non-focal (controls) HHs. Nearly 100% of focal farmers used improved crop varieties and the recommended seed density at the end of the project, along with timely application of fertilizers and insecticides by 80% focal farmers. The crop intensification index indicated that the cropping intensity of focal farmers had increased by ~3% after the project intervention but increased by only 1.6% among non-focal farmers. Average annual income of focal and non-focal farmers was Tk 102,798 and Tk 100,846, respectively at the end-line period, where the contribution of pulses production on the farm income was 45.5 and 43.1 %, respectively.

Following a Mid-Term review (MTR) (2019) recommendation, we developed a new activity on village-based post-harvest processing of pulses. Although de-husking facilities are widely available for rice and wheat, farmers are unable to de-husk mungbean locally. To-date the project has established 21 mungbean de-husking mini mills. With the mini mills farmers are able to de-husk mungbean at Tk 10 per kg and then sell it in the market at Tk 120-140 per kg. Cost Benefit Analysis shows that the margin for per kg mungbean was Tk 70-80. Now farmers are able to sell mungbean at an increased price, impacting their income. Anecdotal evidence suggests there is now a knock-on effect of improved household nutrition and the increased profits from mungbean are now catalysing greater interest and investment in field inputs for the crop unlocking improved production and crop returns.

Component 2 of the project was to evaluate production technologies for dry-season cropping of pulses in saline-free land to increase productivity and profitability. BARI undertook research in Barisal Division in Yrs1 and 2. Following the MTR, agronomic research was re-focused toward the integration of the results into adoptable packages of practices for farmers. An extensive program of demonstrations of dry-season pulses (some at sites common with project LWR/2014/073) showed benefit-cost ratios above 2.0 for production packages on mungbean, relay pea, cowpea, relay grasspea and lentil indicating their profitability. Considering mungbean specifically, in 40 demonstrations conducted over the project duration, the average yield gap between the demonstration package and adjacent farmer control plots was 289 kg/ha indicating a yield advantage of 29%.

In cowpea - a traditional crop of the coastal region - project demonstrations showed that average yields range from 1.24-1.50 t/ha in the region. The cost-benefit ratio over the years was particularly high in Patuakhali which will encourage cowpea adoption as a dry-season cropping option. The planned release of new cowpea cultivars should further boost cowpea production in Southern Bangladesh.

Relay green pea for women: Pea growing households indicate that it is a new crop with gender implications for upland pockets of Barisal. Field pea for green pods taken as a relay crop are considered a low-input cost technology that is easily handled by women-headed HHs. With green pods harvested and marketed weekly by women with good demand at c. Tk 40-50/kg, the technology is attractive to adopt. The Padma Multipurpose bridge opens new potential markets for the produce.

Grasspea production is traditional in the southern region. Project demonstrations showed yield advantages with cultivars BARI Khesari-2 and -3 of ~25% compared to adjacent farmer controls. Despite a benefit-cost ratio for relayed grasspea greater than 2, the crop is risky in the coastal region as establishment is threatened by unusual rainfall and/or flooding in December during germination and early vegetative growth. Improved lines of grasspea are planned for release next year.

Component 3 was to identify wheat germplasm with salinity tolerance adapted to Southern Bangladesh. A benchmark set of 24 wheat lines was established consisting of 12 wheat varieties from Bangladesh, 9 advanced BWMRI breeding lines and 3 putatively salt-tolerant wheat varieties from India and evaluated at 16 field trials in southern Bangladesh. The benchmark lines were ranked for salt tolerance on the basis of the slope of the yield response across seven field sites in the 2017-2018 season. Although the benchmark set of wheat lines was also screened for two key salt tolerance traits; (i) Na+ exclusion and (ii) osmotic stress tolerance, there were no clear relationships between genotypic variation in ranking for either salt tolerance trait or ranking of salt tolerance based on grain yield across a range of saline field environments.

The project investigated the impact of the salt tolerance Nax genes - identified in Australia - on the grain yield of bread wheat grown on saline soils in southern Bangladesh. The key outcome was strong evidence that both Nax genes have the capacity to lower leaf Na+ concentration in locally adapted bread wheat and consequently deliver improved yields in challenging field environments with moderate to high salinity. The genetic backgrounds of the adapted varieties appeared to play a significant role in the expression of these genes. There was a differential response in the phenotypic expression of Nax genes to reduce leaf Na+ levels between the two local varieties selected as recurrent parents and the resultant variation in yield. For example, the average reduction in leaf Na+ concentrations from the third backcrossed lines in the BARI Gom 25 background was about twice that of lines in the BARI Gom 26 background. These same lines yielded on average 10% higher than BARI Gom 25 on moderate to high salinity sites, whereas the lines in the BARI Gom 26 background yielded the same or less than their recurrent parent at these same sites. The contrast in the differential phenotypic expression of Nax2 seen in the two recurrent parents represented in this study highlights the importance of crossing salt tolerance Nax genes into a range of locally adapted high yielding genetic backgrounds in order to identify the greatest potential for improvement in salt tolerance.

Component 4 was to identify germplasm of pulses and forages with tolerance to salinity and water-logging stress. Key advances in this component were made within four UWA PhD theses:

- Md Shahin Uz Zaman (Bangladesh) - PhD (John Allwright Fellow [JAF]) on Waterlogging tolerance at germination in pea - Completed 2019 and returned to BARI
- Edi Wiraguna (Indonesia) – PhD on Waterlogging tolerance in grasspea – Completed 2021 and returned to Indonesia
- Khin Lay Kyu (Myanmar) – PhD (JAF) on Waterlogging tolerance in mungbean - Started 2018 and submitted
- Md Shahin Iqbal (Bangladesh) - PhD (JAF) on Salinity tolerance in mungbean - Started 2020 and on-going

Briefly, lines with improved waterlogging (WL) tolerance were identified in pea, grasspea and mung bean compared to local controls. The inheritance of WL tolerance revealed mostly quantitative gene control, but some marker trait associations were found and candidate genes proposed in pea and mungbean – in association with project CIM-2014-073.

Capacity building of scientists and farmers has been a feature of the project, but this was paused during COVID restrictions.

In conclusion - for saline lands in Southern Bangladesh, technology options offering improved productivity and profitability to farm households were found by the project for a range of pulse crops: mungbean, cowpea, grasspea, green pea and lentil. For saline lands, abiotic stress tolerances were identified for salinity in wheat and for waterlogging in pea, grasspea and mungbean that now need to be introgressed into locally adapted cultivars to realize the potential gains in productivity and profitability.

3 Background

The Government of Bangladesh (GoB) prioritized the coastal zone as the zone most in need of development (MOA 2013). It is home to a population of ~40 M of whom 65% live below poverty line - compared with 40% for the whole country. The coastal zone covers an area of ~1.5 Mha with large areas (~68 %) of flood-prone sometimes saline land (flash-floods, cyclones, and tidal flooding). Canal fresh water is available but as the region has low irrigation availability there is low dry-season agricultural production. The zone covers the most risk-prone areas of the country.

For Bangladesh, ACIAR has shared priorities to improve food security and poverty (targeting MDG 1) in the light of its high vulnerability to the impacts of climate variability. So, the intensification and diversification of climate-resilient rice-based farming systems are high priorities - especially in the vulnerable South of the country. Additionally, we anticipate additional benefits to rural women flowing from the increased wheat and pulse production through increased household dietary diversity, employment opportunities and the stimulation of small businesses.

The Krishi Gobeshona Foundation (KGF) of Bangladesh (a World Bank endowed Trust Fund, funding agricultural research in Bangladesh), which also identified the coastal zone as a priority area, co-funded BARI on-farm research in CIM/2014/076 for three years.

In Southern Bangladesh agricultural activity centres around the annual cropping of monsoonal rice. The harvest of traditional long-duration rice extends from December into February where soils remain wet. Cropping in the dry *rabi* season is economic (Kabir et al 2011), but conditioned by land topography/ drainage, soil salinity and irrigation availability, and as a result household incomes are low with many in poverty.

Rainfed dry-season cropping in such lands is dominated by the pulses – mungbean, grasspea and cowpea – with 292,906 ha grown with traditional agronomy in Barisal Division in 2014 (DAE 2015). In these rainfed lands dry-season cultivation is limited by the profitability of traditional pulse cultivation. Pump availability is a factor limiting dry-season cropping. Nonetheless where some irrigation is possible, wheat is a profitable low risk option and both potato and sunflower are also grown (Kabir and Rawson 2011). In those limited areas where extensive fresh water is available for irrigation *boro* rice is cultivated in the dry season. There is an area of realistically usable rice fallows (medium–high & medium–low land) estimated at between 240,000 and 800,000 ha of non-saline land (Poulton 2011, MoA & FAO 2013). For saline lands, in marked contrast to the above, dry-season options are severely limited by the unavailability of salt tolerant crop cultivars.

To increase farm household income this project aimed to seize the opportunity presented by these extensive dry-season fallow lands for their replacement by more profitable short-duration cropping options. So the overarching research question was: *How can we increase small-holder household incomes through improved productivity and profitability of dry-season crops, on non-saline land and, with pulses and wheat with improved salinity tolerance, on saline land, in Southern Bangladesh?*

There is a mosaic patchiness to land in Southern Bangladesh bringing contrasting land use types – depending primarily on differing micro-topography – into close geographic proximity. Our research strategy had the following elements:

i) Approach by land-use type (saline and non-saline lands)

ii) Capitalise on legacy of such ACIAR projects as CIM/2009/038 and LWR/2005/146

iii) Leverage Australian research expertise in salinity on wheat at Commonwealth Scientific and Industrial Research Organization (CSIRO) and for pulse salinity and waterlogging tolerance at UWA

iv) Continue links with research and dissemination partners forged during CIM/2009/038 with BARI for research and with DAE for dissemination to end-users. (DAE declined to sign an MoU for the project)

v) Supplement funding by KGF for in-country on-farm BARI research and dissemination for three years

vi) Geographic focus for on-farm research and dissemination on pulses in the Barisal Division in the predominantly saline-free Districts of Barguna, Barisal, Bhola, Jhalokati, Patuakhali and Pirojpur.

4 Objectives

Aim: The project aimed to improve smallholder incomes in Southern Bangladesh through improved productivity and profitability of dry-season cropping on non-saline land and, with pulses and wheat with improved salinity tolerance, on saline land.

Under the over-arching question of increasing small-holder household incomes through improved dry-season cropping, critical research questions are:

- In non-saline land which crops - especially pulses - can be grown and how to replace rice fallows and increase production and profitability in Southern Bangladesh?
- What are the needs and limitations to the adoption of the above?
- For saline-affected land can we identify useful variation in salinity tolerance in wheat, key pulses and forages for exploitation in breeding for Southern Bangladesh?

Objectives

1. To understand the context for practice change in dry season cropping in Southern Bangladesh, and establish its evolution as a response to the project.

2. To understand and evaluate production technologies for dry season cropping of pulses in saline-free land to increase productivity and profitability in the Barisal Division in Southern Bangladesh.

3. To identify wheat germplasm with salinity tolerance adapted to Southern Bangladesh.

4. To identify germplasm of pulses and forages with tolerance to salinity and water-logging stress adapted to Southern Bangladesh.

Objectives 3 and 4 were to start with screening protocol development in Australia for transfer to BARI in-country.

Capacity building was embedded within all four objectives to retain focus on the objectives and our principal approach was learning-by-doing.

5 Methodology

Objective 1: To understand the context for practice change in dry season cropping in Southern Bangladesh and establish its evolution as a response to the project.

Activity 1.1 Focus group discussions (FGD) to understand incentives, requirements and obstacles to dry-season cropping

AND Activity 1.3 Assessment of availability & gender dimension of additional labour requirements as options to increase crop productivity and to cut and carry fodder for livestock

The Agrarian Research Foundation (ARF) approached the research for Activities 1.1 and 1.3 together in a single study through a literature review, site visits and holding focus group discussions (FGD) covering 14 upazilas in five districts (Barisal, Barguna, Bhola, Jhalakati and Patuakhali) from April to Sept. 2018. A total of 24 FGDs were held, 20 with farmers, 2 with mungbean and grasspea traders, 1 with officers and scientists, and 1 with trade association.

Activity 1.2 Baseline and final surveys of households in targeted project region

Bangladesh Agricultural University (BAU) (Professor Taj Uddin - Agriculture Economics Dept) completed a baseline survey of 500 households (HH) of farming practices and livelihoods in both saline and non-saline affected areas of Southern Bangladesh with student assistance (See Section 3.2) in 2019 (Uddin et al. 2019; Islam 2019). The survey gathered data on household socioeconomic profile; farming practices and crop intensification (especially wheat and pulses); information related to employment & household income/ expenditure; and intra-household labour allocation, livelihood status; and finally, constraints to possible opportunities/solutions.

In 2020/21 the same group conducted another survey to understand the context for practice change in dry season cropping in non-saline areas of Southern Bangladesh, and to see its evolution in response to project intervention. A total of 240 farmers, comprising 120 focal (hosted demonstrations) and 120 control growers, were investigated following stratified sampling in non-saline areas of four districts (Patuakhali, Barisal, Jhalokathi, and Barguna).

Activity 1.4 Profitability assessment of new technologies based on on-farm data by UWA/BARI

Profitability assessments by gross margin analysis and benefit-cost ratio were undertaken on the demonstrations each year by BARI scientists and the data are intercalated with the relevant agronomic results under Activity 2.5.

Activity 1.5 Capture household 'change stories' by tracking changes following demonstrations in Objective 2

The NGO - Poverty Eradication Program (PEP) developed two change stories in the Barisal Division during the extension period by report and video output. The first story was 'An overview of Salt-tolerant wheat cultivation in coastal fallow lands in Bangladesh'. The second was 'Mungbean de-husking mini mill: A powerful tool for farmers' solutions'.

NEW Activities 1.6 Piloting of mini mills to dehusk mungbean in Barisal Division by PEP and Activity 1.7 Installation of 20 additional mini mills at existing rice mills with PEP

A recommendation of the Mid-Term Review (MTR) was to develop a new activity on village-based post-harvest processing for pulses.

The project started mini mill activity under ARF by visiting a lentil de-husker mini mill in Bhola developed in ACIAR project (LWR/2005/001). The machine was found less appropriate for mungbean de-husking than for other pulses, like lentil. Considering this situation, the project contracted ARF to establish three pilot mungbean de-husking machines to understand the impact of mini mills at the community level.

During the PEP's first contract with the project, the project introduced a total of 19 mungbean de-husking facilities at the community level in mungbean growing areas of the districts of Patuakhali (10 mini mills) and Barguna (9). Further mini mills (10) are being introduced by PEP during the extension period. This is not yet completed.

Objective 2: To understand and evaluate production technologies for dry season cropping of pulses in saline-free land to increase productivity and profitability in the Barisal Division in Southern Bangladesh

Activities 2.1 to 2.4 were primarily conducted by researchers of the BARI Pulses Research Centre (PRC) mostly on-farm, with some trials conducted on-station. Following the MTR/Annual Planning Meeting in 2019, Objective 2 research was re-focused - where possible - into production packages for demonstration (Activity 2.5) to ensure their potential benefits flow to growers. Only experimentation on mungbean weed control and pest management continued in Yrs3 and 4 because improvements over standard practice had not been identified. Demonstrations - under Activity 2.5 - were implemented each year of the project and conducted by BARI On-Farm Research Division (OFRD) with KGF funding in Yrs1 through Yr4, until 2022 (Yr5) when they were run by PEP.

Activity 2.1. Evaluation on-farm of improved mungbean technologies (cultivars & management practices)

An Adaptive Trial of mungbean varieties to evaluate the performance of released mungbean varieties in Barisal Division and to decide which to use for on-farm demonstration was conducted as a field experiment during late *rabi* season at three sites in Yrs1 and 2. Eight mungbean varieties viz. BARI Mung series -1 to -8 were included in 2018, while in 2019 both BINA Mung 5 & 8 were added.

Effect of sowing date: An on-farm study of sowing date was conducted on farmers' fields at two sites during late *rabi* season of Yrs 1 and 2. Sowing dates were from early January until March 1st. The trial was then discontinued, but January sowing was incorporated into the demonstration package (Activity 2.5).

Effect of sowing method: An experiment to compare sowing methods in mungbean was conducted at three southern-belt farmer field sites in Yr1 and one site in Yr2. Four sowing methods were used: T_1= Broadcast (maintaining optimum population 36 pl / $m^{2)}$, T_2= Line sowing (30 cm x 7-8 cm), T_3= Line sowing (40 cm x 7-8 cm) and T_4= Conventional tillage/farmers (broadcast with variable seed rate) practice. Seed drilling was incorporated into the production package for mungbean demonstration (See 2.5) and research on the issue terminated.

Weed control: Weed control options were examined on a farmer's field using line sowing (30 cm-apart) at Rahmatpur, Babuganj, and Barisal in Yr1 and repeated at Rahmatpur in Yr2. The trial comprised four weed control treatments i) Control – No weeding ii) One hand weeding, iii) two hand weedings, and iv) Whip Super^{TM} (Fenoxaprop-p-ethyl) herbicide application @1.5ml/l water at 20 DAE. As the trial did not reveal any improvement upon farmer methods, in Yrs3 and 4 a further trial on weed management was conducted in farmers' fields at Babuganj, Barisal with five treatments: W1= Control; W2= One hand weeding 20 days after emergence (DAE); W3= BARI weeder (hand-operated row hoe) at 20 DAE; W4= W2 + BARI weeder at 40 DAE; W5= Spraying of Weednil^{TM} (Quizalofop-p-ethyl) at 20 DAE@ 1.5 ml/l.

Integrated pest management (IPM): The effectiveness of IPM approaches using botanicals, synthetic insecticides and blue sticky traps was evaluated at RARS Rahmatpur, Barisal during late *rabi* Yr1 and 2. As the cost of the treatment was greater than farmer insect control by Imidacloprid, another approach to pest management was taken in Yrs 3 & 4 with micro and macro-nutrients in trials at Ishurdi and Rahmatpur (Yr3 only). Treatments were: 1, Untreated control, 2, Microvit 80 WP @ 1 g/l, 3, Thiovit 80 WG

@ 2 g/l; 4, Nutra-phos-24 @ 3 g/l; 5, McChili @ 0.35 g/l + Solubor @ 1 g/l; and 6, Muriate of potash @ 10 g/l.

Activity 2.2. Cultivar identification and evaluation on-farm of improved cowpea technologies

Germplasm evaluation: Cowpea germplasm was introduced from the International Institute of Tropical Agriculture (IITA) to BARI during the previous project CIM-2009-038 and preliminary evaluation undertaken. During late *rabi* seasons of Yr1 through Yr4, eight selected lines and a local check variety (BARI Felon-1) were assessed at RARS Rahmatpur, Barisal. Additionally, in Yr3 the trial was grown at RPRS, Madaripur, and in a farmer's field at Noakhali.

During the project a further tranche of 350 germplasm accessions was introduced from IITA. By Yr4 the 30 best lines were grown at RARS, Rahmatpur in a replicated trial.

Production practices: Existing best practices for cowpea production were evaluated on-farm in the coastal region in Yrs1 and 2. The management package comprised variety (BARI Felon 1), fertilizer application (NPKS 20-40-20-10 kg/ha with nitrogen top dressing) and a seed rate of 70 kg/ha. Following discussion during the MTR/annual planning meeting, the on-farm evaluation of cowpea practices was terminated, and the production package transferred into project demonstrations (Activity 2.5) from Yr3.

Activity 2.3. Cultivar identification and evaluation on-farm of improved grasspea technologies.

Grasspea germplasm was introduced from the International Center for Agricultural Research in the Dry Areas (ICARDA) to BARI during the previous project CIM-2009-038 and preliminary evaluation undertaken. Germplasm evaluation of eight selected lines was undertaken at RARS, Rahmatpur, Barisal from Yr1 to Yr4, when testing was extended to RPRS, Madaripur.

Production practices: An on-farm trial of grasspea was conducted to evaluate improved management practices for the relay sowing with T. *aman* rice at two locations Barisal and Jhalokathi districts during rabi season of Yrs1 and 2. The production package comprised improved variety (BARI Khesari 3), fertilizer application (NPKS at 20-40-20-10 kg/ha with nitrogen top dressing), seed rate of 75 kg/ha and 30 cm stubble height retention of T. aman rice on 1 ha at each location. Following discussion during the MTR/2019 annual planning meeting, the production package was used in project demonstrations (Activity 2.5).

Activity 2.4: Evaluation on-farm of relay-sowing of green pea and lentil

Lentil production technology: A package for lentil production using relay sowing was produced for Western Bangladesh in the previous project (CIM-2009-038). In Yr1 a field trial was conducted on relay-sown improved management practices of lentil in char-land (actively eroding/accreting riverine deposits) in Barisal with 16 farmers on 2 ha. Seeds of BARI Masur-6 @ 50 kg/ha were hand-broadcast in the standing field of T. *aman* rice on 25-27 November 2017. In the following two *rabi* seasons the on-farm trial was conducted with the lentil relay sowing package at three villages in Barisal. The improved management practices included BARI Masur 8 variety, fertilizer package of 14-20-20-10 kg/ha of NPKS with Nitrogen top dressing, seed rate of 60 kg/ha and 30 cm stubble height retention of T. *aman* rice. As the results were promising, the practices were integrated into project demonstrations (Activity 2.5).

Pea production technology: This on-farm study was undertaken to evaluate pea as a relay crop with T. *aman* rice in Babuganj, Barisal for green pod production during *rabi* season of Yrs 1 and 2. The relay sowing package included improved variety e.g. BARI Motor- 3 (dual-purpose grain and green pod use), fertilizer of 14-20-20-10 Kg/ha of NPKS with one N top dressing, seed rate of 90 kg/ha and retention of 30 cm stubble height of T. *aman* rice. As with lentil, the package was moved into project demonstrations.

Activity 2.5: Demonstrations of profitable production technology of mungbean, cowpea, grasspea and lentil and green pea

Mungbean**:** The demonstrated technology package comprised line sowing (30 cm apart) at 25 kg seed/ha of cultivar (BARI Mung 6) with fertilizer (NPKS 20-40-20-10 kg/ha). Insecticide was applied (Volume Flexi @ 0.5ml/l two times [before and 15 days after flowering]) to control thrips and pod borer. In Yr1 (14 demonstrations) and Yr2 (12) the demonstrations were one ha each spanning several farmers' land and at harvest demonstration yield was compared with nearby farmer plots. In Yrs 3 (7 demos) and Yr4 (7) the size of individual block demonstrations was increased to 3-5 ha with the number of sites being reduced to one each in Barguna, Barisal, Bhola, Jhalokati, Khulna, Patuakhali and Pirojpur. Gross margin analysis and the benefit cost ratio (BCR) were calculated for the demonstrations based on a price of Tk 50/kg mungbean in 2019 and 2020 and Tk 60/kg in 2021.

Cowpea: Block demonstrations were conducted in the *rabi* season sown from Yr1 through Yr4 in Barguna, Bhola and Patuakhali. The demonstrations were like those above on mungbean. The technology package comprised line sowing by PTOS of cultivar (BARI Felon 1) with fertilizer (NPKS 20-40-20-10 kg/ha). Insecticide was applied to control pod borer, as necessary. Gross margin analysis and BCR were calculated for the demonstrations based on a unit price of 40 Tk/kg in Bhola and 35 Tk/kg elsewhere for cowpea grain.

Grasspea**:** The demonstration package included either BARI released variety BARI Khesari-2 or BARI Khesari-3 sown during last week of November into the standing rice crop as relay cropping 10-15 days before the rice harvest. Fertilizer @ 20-40-20-10 kg/ha of NPKS was applied before sowing basally and N was top-dressed at 20-25 DAE. A seed rate of 75 kg/ha was used. T. *aman* rice was harvested retaining ~30 cm stubble height. Grasspea demonstrations were in Barisal, Bhola, Barguna, Patuakhali and Pirojpur, with Jhalokati added in Yr4. Gross margin analysis and BCR were calculated for the demonstrations based on a unit price 40-45 Tk/kg grasspea depending on year and region.

Lentil: Demonstrations of an improved production package were undertaken from Yr 1 to Yr4 on upland soils in Barisal and Bhola. The technology package consisted of BARI-released lentil variety BARI Masur 7 sown in November at the rate of 45 kg/ha with fertilizer application 14-20-20-10 kg/ha of NPKS. Generally, the pilot areas were 1.3 ha in size incorporating the land of several (5-10) farmers. As lentil and pea are uncommon in Southern Bangladesh it was difficult to find neighbouring fields for comparative purposes. Gross margin analysis and BCR were calculated for the demonstrations with a unit price of 50 Tk lentil and dry pea 50 Tk/kg.

Pea: Demonstrations of pea were conducted each season from Yr1 to Yr4 in plots of ~1.3 ha involving several farmers' land in Barisal. They comprised green-pea variety BARI Motor 3 sown in late Nov/early December at the rate of 45 kg/ha with fertilizer application 14-20-20-10 kg/ha of NPKS.

Objective 3: To identify wheat germplasm with salinity tolerance adapted to Southern Bangladesh.

Activity 3.1. Establishment of set of lines and their evaluation to benchmark current and future wheat germplasm in two polder sites with LWR/2014/073

A benchmark set of wheat lines from BARI was established to develop salt tolerance ranking parameters to eventually evaluate other wheat germplasm; and secondly, to be used to establish appropriate field trial design and soil characterisation methodology for the accurate assessment of salt tolerance and performance in saline field trials. The benchmark set of 24 wheat lines consisted of wheat varieties previously and currently grown in Bangladesh (BARI Gom varieties), potential future releases (advanced breeding lines from BWMRI) and putatively salt-tolerant wheat varieties (KRL lines) from India.

The benchmark set of wheat lines was evaluated at a total of 16 field trails at 8 locations over two years in southern Bangladesh (Table 5.3.1). Two trials were established at each location, typically one inside a polder the other outside, to provide the capacity for evaluation of wheat lines under different salinity levels at a given site. A trial design was developed and used to account for the typical heterogeneity in the distribution soil salinity. Each trial was set out in four continuous blocks (reps) in a 6 row by 4 column design for each block, which allowed for randomisation of lines within block and latinization within column. A unique randomisation was used for each trial.

Table 5.3.1. List of field trial sites, with GPS coordinates and sowing and harvest dates where 24 benchmark wheat lines were evaluated over two seasons, (a) 2017-2018 and (b) 2018-2019. Note: three locations used in the 2017-2018 season are not listed below as the yield data was not used due to very low heritability.

(a) 2017 – 18 season

Site name	Location	GPS coordinates	Sowing date	Harvest date
Barisal (II)	Manikkathi, Uzirpur	22.8065 E, 90.2935 N	26/12/2017	05/04/2018
Patuakhali-I	Diaramkhola, Kolapara	22.3578 E, 90.3376 N	27/12/2017	05/04/2018
Patuakhali-II	Hazipur, Kolapara	21.8983 E, 90.1422 N	30/12/2017	07/04/2018
Satkhira-I	ARS, Benarpota, Satkhira	22.4458 E, 89.0621 N	26/12/2017	04/04/2018
Satkhira-II	Harodda, Satkhira sadar	22.6690 E, 88.9502 N	27/12/2017	04/04/2018
Khulna (II)	Sachibunia, Botiaghata	22.7812 E,89.5362 N	22/12/2017	29/03/2018
Noakhali (II)	Atkapalia, Subornochar	22.7759 E, 91.3220 N	21/12/2017	29/03/2018

(b) 2018 – 19 season

Site name	Location	GPS coordinates	Sowing date	Harvest date
Patuakhali-I	Diaramkhola, Kolapara	22.3578 E, 90.3376 N	16/12/2018	23/03/2019
Patuakhali-II	Hazipur, Kolapara	21.8983 E, 90.1422 N	17/12/2018	28/03/2019
Satkhira-I	ARS, Benarpota, Satkhira	22.4458 E, 89.0621 N	15/12/2018	28/03/2019
Satkhira-II	Harodda, Satkhira sadar	22.6691 E, 88.9502 N	29/11/2018	18/03/2019
Khulna-I	Koyra, Khulna	22.3311 E, 89.3082 N	28/11/2018	07/04/2019
Khulna-II	Dacope, Khulna	22.3726 E, 89.2728 N	28/12/2018	07/04/2019

Salinity monitoring and mapping was completed on every field plot at two times during the season; at sowing and at anthesis using both a portable dual EM meter and EC measurements on soil cores. Soil cores were extracted to a depth of 50 cm in two depth intervals (0-25cm and 25-50cm) from every plot in each trial. Grain yield was analysed via linear mixed models (LMM's) incorporating terms to account for trial design and utilising plot salinity levels and plant establishment as covariates when significant. Analysed yield data were used to assign line rankings in response to salinity and to establish the relationship between salinity and yield.

Training workshop to improve the capacity for experimental design and analysis

An Experimental Methods Workshop on wheat and pulses involving presentations and discussions was undertaken at BARI in Gazipur in July 2018. For the wheat component of this project, the workshop covered an introduction to salinity tolerance traits, general guidelines for salinity tolerance screening, screening methods and protocols for the three key salt tolerance traits and trial design and soil characterisation principles and methods for running field trials in saline environments.

Activity 3.2 Screening protocols and equipment established at BARI Gazipur

Supported hydroponics infrastructure used for screening wheat and other species for salt tolerance traits was procured and assembled by the CSIRO team in Australia and sent to BARI in Gazipur (March 2019). A comprehensive PDF detailing all the equipment and methods required to construct and run the supported hydroponics infrastructure was also developed by CSIRO and sent to the BARI Plant Physiology Team to assist in the assembly, testing and experimental use of this phenotyping infrastructure.

Activity 3.3 Germplasm screening for salinity tolerance traits

Using the screening infrastructure and experimental protocols delivered to BARI (see Activity 3.2) a series of supported hydroponics salt tolerance screening experiments evaluating a collection of 150 BARI wheat lines, was completed by the Plant Physiology team at BARI (Gazipur) between November 2019 and March 2020. Plants were grown in 150 mM NaCl for 3 weeks for the purpose of evaluating Na^+ exclusion capacity and leaf K^+ concentration. While the ion analysis is yet to be completed, a range of growth parameters (plant FW, shoot height and DW, root length and DW) were assessed and analysed.

Activity 3.4 Introgression of improved salinity tolerance into locally adapted wheats

The focus for this activity was on the salt tolerance *Nax* genes (*Nax1* & *Nax2*) previously discovered and characterised by Munns and James at CSIRO. These genes in an adapted Australian wheat background together with marker information were transferred to Dr Yousuf Akhond (Biotechnology Division, BARI) just prior to the commencement of this project, who started a breeding and backcrossing program to introgress them into two recent Bangladeshi wheat varieties, BARI Gom 25 and BARI Gom 26.

72 $BC_3F_{3:5}$ families fixed for either *Nax*1 or *Nax2* (36 from each BARI Gom background) were developed and subsequently assessed in un-replicated small plots at BARI in Gazipur, for the purpose of seed bulking and assessing agronomic features, primarily height and phenology, as additional criteria to aid and refine selection of lines for field evaluation. Seed from these 72 $BC_3F_{3:5}$ families was also transferred to CSIRO in Australia, where a large phenotypic screen using supported hydroponics was completed to validate the Na^+ exclusion status of these lines. This was complemented by a repeated genotypic screen using new optimised markers by Dr Yousuf Akhond, to confirm the identification of homozygous (fixed) lines for *Nax1* and *Nax2* in both backgrounds.

A series of field trials to evaluate the salt tolerance of a subset of the recently developed and validated fixed *Nax* breeding lines in BARI Gom 25 & 26 backgrounds was completed at a total of nine locations over three field seasons (2019 – 2022) in southern Bangladesh. 20 wheat genotypes were evaluated including 12 *Nax1* or *Nax2* advanced breeding lines, parental lines (BARI Gom 25, BARI Gom 26) and six check lines from the Benchmark set. These field trials were undertaken in different trial sites at three main locations, at Patuakhali, Khulna and Satkhira. Trial design was a slight variation to that described previously in Activity 3.1. Briefly, genotypes were randomised uniquely in each trial following a latinised 4 x 5 RCB design with four replications (blocks). Soil salinity was measured on all plots across all trials at sowing and at anthesis by using a portable dual EM (ECa) device and soil sampling (ECe) to a depth of 50 cm in two depth intervals (0-25cm and 25-50cm). Grain yield was analysed via linear mixed models taking into account trial design, crop establishment and plot salinity levels as covariates when significant.

Activity 3.5. Screening of wheat lines for salinity tolerance traits at CSIRO (Australia)

To identify wheat germplasm with salinity tolerance adapted to Southern Bangladesh, screening two key wheat collections for salt tolerance traits was completed by CSIRO in Australia:

- ‘Benchmark set’ of 24 wheat lines
- 150 diverse BARI wheat lines

An additional set of screening experiments for Na^+ exclusion was completed by CSIRO on 72 $BC_3F_{3:5}$ families fixed for either *Nax*1 or *Nax2*, which is summarised in Activity 3.4.

‘Benchmark set’ of 24 wheat lines

The ‘benchmark set’ of 24 wheat lines were sent to CSIRO and bulked through Australian quarantine processes at CSIRO, were assessed for two key salt tolerance traits: (i) leaf Na^+ exclusion, K^+ concentrations and K:Na ratio, and (ii) Osmotic stress tolerance.

Leaf Na^+ and K^+ concentrations were measured after growing wheat seedlings in supported hydroponics in four 40-L trays containing 150 mM NaCl in a controlled environment chamber. Salt concentration was increased incrementally and supplemental Ca^{2+} ($CaCl_2$) was added. After 10 d in 150 mM NaCl, the blade of leaf 3 was harvested and analysed for Na^+ and K^+ by an Inductively Coupled Plasma – Atomic Emission Spectrometer. Ion data were statistically analysed using a linear mixed model and Best Linear Unbiased Estimates (BLUEs) were calculated.

Screening for Osmotic tress tolerance was via an automated imaging platform based at CSIRO called ‘Phenopod’ - to non-destructively estimate leaf area and growth rates of wheat lines grown in saline and control conditions. Osmotic stress tolerance parameters were assessed via two different methods; (i) ratio of relative growth rates (RGR) of salt treated versus control seedlings, and (ii) ratio of shoot biomass of salt treated versus control seedlings at the conclusion of the experiment. Wheat seedlings were in large pots filled with a soil mixture consisting of loam compost, coarse sand, and perlite which allowed for both water holding capacity and good drainage. Treatments were 150 mM NaCl (added incrementally) and control (no salt). Two large experiments were conducted in a naturally lit glasshouse where pots were scanned by passing through an RGB imaging station to capture 18 images of each seedling on a series of 20^o rotations, from which digital volume was calculated. All seedlings were imaged four times for each experiment at 6, 9, 13 and 16 days after the commencement of the salinity treatment. Relative growth rates (RGR) were calculated from the increase in the number of pixels over the four sets of RGB scans. At the end of the experiments, shoots were harvested and dried to calculate the ratio of shoot biomass from salt-treated versus control was calculated.

150 diverse BARI wheat lines

150 wheat lines representing genetic diversity in the BARI (now BWMRI) wheat breeding program were selected by BWMRI wheat breeders, sent to CSIRO (February 2019) and successfully multiplied through the Australian quarantine protocol at CSIRO. This population was also screened for two key salt tolerance traits: (i) leaf Na^+ exclusion and K^+ concentrations and (ii) Osmotic stress tolerance.

A series of five large successive Na^+ exclusion screening experiments were completed, where seedlings were grown in supported hydroponics at 150 mM NaCl over three weeks using the same methodology as described above. Harvested leaves were analysed for Na^+ and K^+ by an Inductively Coupled Plasma – Atomic Emission Spectrometer

Screening for Osmotic tress tolerance was undertaken using an automated glasshouse imaging infrastructure located at The Plant Accelerator (TPA) an Australian Plant Phenomics Facility (APPF) in Adelaide. A single large screening experiment commenced in September 2020, consisting of 149 wheat lines grown in 1056 2.5L pots (3 – 4 replicates per line) in climate-controlled glasshouses, with two salinity levels, 0 mM NaCl (control) and 150 mm NaCl. Salinity treatments commenced 14 – 17 days after planting (DAP). Experimental design was an unequally replicated, latinized, incomplete block design. Automated non-destructive imaging to determine projected shoot areas (PSA) was completed daily on all plants between 10 – 30 DAP using RGB cameras from three camera views. At the completion of the experiment the shoots of all plants were harvested for dry weight measurements. A range of growth and growth rate metrics (absolute growth rates - AGR, relative growth rates - RGR) from the non-destructive imaging protocol were calculated and statistically analysed to give line (genotype) predictions for each growth metric using a linear mixed model approach.

Objective 4: To identify germplasm of pulses and forages with tolerance to salinity and water-logging stress adapted to Southern Bangladesh.

Activity 4.1 Developing germplasm screening protocols for salinity and water-logging for mungbean, cowpea and grasspea at UWA. Identification of traits responsible for tolerance at UWA (After the identification of extremes for tolerance in #4.2 selected accessions

And Activity 4.2 Screening of pulse germplasm to identify sources of stress tolerance

At UWA we developed stress screening protocols with post-graduate students (See 8.2 Capacity impact) for tolerance to waterlogging in pea, mungbean, grasspea and lentil and to salinity in mungbean. Following the transfer of protocols to BARI at the July 2018 methodology workshop, systematic screening of pulses for waterlogging tolerance (using the project-imported electrical conductivity metre) and salinity began in-country. We introduced to BARI germplasm of cowpea from IITA, and grasspea from ICARDA; while the World Vegetable Centre mini-core collection of mungbean was already available. Screening of the mungbean mini-core collection for both tolerances was in cooperation with project CIM-2014-079 (*Establishing the international mungbean improvement network*). The approach taken with each crop was to establish the abiotic stress screening protocol in a pilot experiment with a few genotypes subjected to a wide range of stress treatments, and then building on the pilot trial to proceed with screening diverse germplasm to identify sources of stress tolerance, and in mung bean also to understand its inheritance through Genome-Wise Association studies (GWAS) using genomic data on the mini-core from WorldVeg.

Activity 4.3 Field evaluation of germplasm of salt tolerant fodder options

Capitalising on the earlier evaluation of pasture legumes for salinity and waterlogging tolerance in Australia, we aimed to import tolerant lines to Bangladesh. Note that we added waterlogging tolerance to the aims in view of the finding of ARF under Objective 1 of the importance of water logging to annual legume production in Bangladesh. The tolerant lines were balansa clover (*Trifolium michelianum*) cv. Paradana (tolerates waterlogging and mild salinity), Messina *(Melilotus siculus*) cv. Neptune (tolerates both waterlogging and salinity), and burr medic (*Medicago polymorpha*) cv. Scimitar (tolerates moderate salinity and transient waterlogging only) - each with associated rhizobium.

Taking an alternative approach to fodder production a field study was conducted to evaluate existing pulse crops as forages. An experiment was conducted in a farmer’s field in Barisal in the rabi season 2020-21 using grasspea (cvs BARI Kheshari-2 and BARI Kheshari-3) and field pea (BARI Motor-3).

New Activity 4.4 UWA completion of experimental work for: 1. Improved protocol for genetic modification procedures; 2. Standard operating practice (SOP) for clonal propagation of mungbean and 3. SOP for year-round accelerated Single Seed Descent (aSSD)

Protocol for genetic modification: To test the effect of light spectra on in vitro regeneration and transformation efficiency, first we established a conventional in vitro regeneration protocol based on Sonia et al. (2007) with modifications and an effective concentration of hygromycin (Hyg) for selection of transformed shoots. The shoot regeneration medium was tested with and without activated charcoal. An effective concentration of Hyg for the selection of transformed shoots was determined by culturing control nodal cotyledon explants (non-transformed) on shoot regeneration medium containing different concentrations of Hyg (0, 25, 50 & 75 mg/L) and the addition of cefotaxime (0 & 300 mg/L). To establish a gene transfer protocol for mung bean (which can also be used for gene editing) purposes, after the establishment of the regeneration protocol and antibiotic protocol, we tested different light spectra during Agrobacterium co-culture during transformation. Lights tested were 1. conventional fluorescence, 2. enriched in the red region of the spectrum by Heliospectra DYNA system and 3. spectra enriched in the blue region. Transformation was assessed using GFP expression and calculated as the number of explants showing green fluorescence shoots out of the total number of explants treated.

Clonal propagation: To develop a protocol for clonal propagation of mungbean, we studied three mung bean cultivars (Celera II-AU, Jade and BARI Mung-6) and one black gram (Onyx-AU) cultivar. We investigated (1) growth environmental conditions of the mother plant (13 h v. 16 h photoperiod), (2) the plant development stage at the time of cloning and, (3) the type of plant hormone (auxins: indole acetic acid (IAA), naphthalene acetic acid (NAA) and indole-3-butyric acid (IBA)) to induce adventitious rooting.

accelerated Single Seed Descent (aSSD): To determine the most appropriate conditions for early floral onset and shorter generation time in mung bean and black gram, plants of four cultivars (as above) were grown in a range of environments with different physical conditions. Environment 1 (ENV1) is the control (in-season growing) (Natural light, intensity 1400 µmol m^{-2} s^{-1} (at midday), photoperiod 13.5 - 13 h); and ENV2 designed for early flowering (far red-enriched LED light, 500 µmol m^{-2} s^{-1}, 13 h); and a long day ENV3 (as ENV2 but long photoperiod [16 h] = negative control).

6 Achievements against activities and outputs/milestones

Objective 1: To understand the context for practice change in dry season cropping in Southern Bangladesh, and establish its evolution as a response to the project

Activities	Outputs/ Milestones	Applications of outputs
Activity 1.1 Focus group discussions to understand incentives, requirements and obstacles to dry-season cropping Activity 1.3 Assessment of availability & gender dimension of additional labour requirements as options to increase crop productivity and to cut and carry fodder for livestock	Combined report on Activities 1.1 and 1.3 completed and available.	Researchers (Obj. 2-4) design technology options to seize opportunities for/ mitigate obstacles to increased dry-season cropping. Researchers in Obj. 2-4 design gender-sensitive technology options and employ gender appropriate training approaches. Specifically: (i) mungbean was identified as a woman-friendly crop with most operations done by women; (ii) Farmers are receiving a poor return of their mungbean produce due to unavailability of de-husking facilities for mungbean at the village level (increased project emphasis then made on community dehusking mini mills); (iii) Importance of water logging to pulse production (increased project emphasis under Objective 4 on waterlogging tolerance); (iv) Decreasing trend in area under grasspea (reflected in project grasspea activities); (v) Gender implications of field pea harvested and marketed for green pods (increasing project emphasis on green pea pods as opposed to dry grain use)
Activity 1.2 Baseline survey of households in targeted project region by BAU Endline survey of households	Report available on baseline survey (Uddin et al. 2019; Islam 2019) Report available on endline survey	Researchers have improved understanding of household dynamics. Initial adoption quantified
Activity 1.4 Profitability assessment of new technologies based on on-farm data	Profitability reported under demonstrations annually in Ann. Reports	Researcher awareness regarding division of labour (above) and impact of increased productivity/profitability to drive income-related changes at household level
Activity 1.5 Capture household ‘change stories’ by tracking changes following demonstrations in Objective 2	Activity completed	Two change stories completed by report and video in Barisal Division by PEP: One on wheat and salinity and the other on community mini mills for mungbean
New Activity 1.6 Piloting of mini mills to dehusk mungbean in Barisal Division by PEP	Activity completed	Twenty-one mini mills installed in Patuakhali and Barguna. 10 more to be installed
New Activity 1.7 Installation of 20 additional mini mills at existing rice mills with PEP	Activity on-going with PEP	

Objective 2: To understand and evaluate production technologies for dry season cropping of pulses in saline-free land to increase productivity and profitability in the Barisal Division in Southern Bangladesh

Activities	**Outputs/ milestones**	**Applications of outputs**
Activity 2.1. Evaluation on-farm of improved mungbean technologies (cultivars & management practices) APSIM mungbean growth model validation with LWR/2014/73	Reported in Project Annual Reports: • Adaptive trial of mungbean varieties (Yrs1 & 2) • Effect of sowing date (Yrs1 & 2) • Effect of sowing method (Yrs1 & 2) • Weed control (Yrs1 to 4) • Integrated pest management (IPM) (Yrs1 to 4) (Hossain et al. 2020) APSIM mungbean growth model not explored	Results incorporated into demonstration package (see Activity 2.5): • Cultivars: BARI Mung 6 followed by BARI Mung 5&7 • Early Sowing in January • Seed drilling/line sowing
2.2. Cultivar identification and evaluation on-farm of improved cowpea technologies: On-station germplasm evaluation Evaluation on-farm of improved technologies	Reported in Project Annual Reports: Germplasm evaluation (Yrs1 to 4) On-farm evaluation of technology (Yrs1 and 2)	Superior lines identified for cultivar release and seed multiplication Technologies incorporated into demonstration package (Activity 2.5)
2.3. Cultivar identification and evaluation on-farm of improved grasspea technologies: On-station germplasm evaluation Evaluation on-farm of improved technologies	Reported in Project Annual Reports: Germplasm evaluation (Yrs1 to 4) On-farm evaluation of technology (Yrs1 and 2)	Superior lines identified for cultivar release and seed multiplication Technologies incorporated into demonstration package (Activity 2.5)
2.4: Evaluation on-farm of relay-sowing of: • Green pea • Lentil	Reported in Project Annual Reports: Green pea and Lentil - On-farm evaluation of technology (Yrs1 to 4)	Technologies incorporated into lentil and pea demonstration packages (Activity 2.5)

2.5: Demonstrations of profitable production technology of mungbean, cowpea, grasspea and lentil and green pea Brochure preparation Demonstrate pulse post-harvest processing at field days	Reported in Project Annual Reports: Demonstrations, farmer training and field days on mungbean, cowpea, grasspea and lentil and green pea Brochures in Bangla available on improved production technologies on mungbean, relay grasspea, relay green pea and mini mill dehusking (in Bengali and English) Twenty-one dehusking mills installed (10 more planned during extension)	Households adopt new technologies Households gain improved return for their mungbean and invest in inputs to increase production
New activity 2.6 Conclusion of field trials by BARI on mungbean, cowpea, and garden pea in final rabi season	Report on field trials by BARI on mungbean, cowpea, and garden pea available	Information will be used by BARI PRC program and for extension
New activity 2.7 Demonstrations of best practices in mungbean, cowpea, and garden pea in final rabi season by PEP in final season	Report on demonstrations on mungbean, cowpea, and garden pea available	
New activity 2.8 Demonstrations (5) of best practices in mungbean in mini mill catchment areas by PEP in final season	Report on demonstrations on mungbean available	

Objective 3: To identify wheat germplasm with salinity tolerance adapted to Southern Bangladesh

Activities	**Outputs/ Milestones**	**Applications of outputs**
Activity 3.1. Establishment of set of lines and their evaluation to benchmark current and future wheat germplasm in two polder sites with LWR/2014/073 Training workshop to improve the capacity for experimental design and analysis	A set of common 24 check lines for benchmarking established. Report available on evaluation of 24 benchmark wheat lines on a range of saline field trials over two seasons and development of salt tolerance ranking (James et al., 2022b) Experimental Methods Workshop successfully completed.	Ranked wheat lines to be used to benchmark newly developed putatively salt tolerant advanced wheat lines (Activity 3.4, 3.5) Field evaluation protocols including design, soil characterisation and analysis components to be used in the evaluation of future advanced breeding lines. Training outputs used for Activities 3.2, 3.3 and 3.4
3.2 Screening protocols and equipment established at BARI Gazipur	Supported hydroponics equipment established at BARI (Plant Physiology dept) Detailed methods document available on protocols and equipment required for controlled environment screening of salt tolerance traits	Equipment and method protocols used for Activity 3.3 and available for future screening activities

3.3 Germplasm screening for salinity tolerance traits	Reported in Project Annual Reports: Report available on germplasm identified and ranked for salinity tolerance (growth) trait (Imrul et al.)	Identified sources of salinity tolerance traits to be incorporated in BWMRI breeding program (with outputs in Activity 3.5)
3.4 Introgression of improved salinity tolerance into locally adapted wheats	Report available on the introgression of salt tolerance *Nax* genes into adapted Bangladeshi wheats and subsequent evaluation in saline field trials in southern Bangladesh (James et al. 2022a)	Developed and validated salt tolerant lines in BARI Gom 25 background to be crossed with best adapted advanced breeding lines for eventual release by BWMRI breeders
3.5. Screening of wheat lines for salinity tolerance traits at CSIRO (Australia): a. Na+ exclusion (controlled environment, seedling stage hydroponics experiments with detailed ion analysis. b. Osmotic stress tolerance (4 – 6 weeks) wheat seedling growth experiments in controlled environment chambers.	Reported in Project Annual Reports: Novel genotypic variation for both Na+ exclusion and osmotic stress tolerance identified in BWMRI wheat lines	Identified sources of salt tolerance traits to be incorporated in a targeted breeding program by BWMRI wheat breeders.

Objective 4: To identify germplasm of pulses and forages with tolerance to salinity and water-logging stress adapted to Southern Bangladesh.

Activities	**Outputs/ Milestones**	**Applications of outputs**
Activity 4.1. Developing germplasm screening protocols for salinity and water-logging for mungbean, cowpea and grasspea at UWA. Identification of traits responsible for tolerance at UWA (After the identification of extremes for tolerance in #4.2 selected accessions	Reports available on optimized screening protocol for water-logging tolerance in mungbean (Khin Lay et al. 2021), lentil (Wiraguna et al. 2017), pea (Zaman et al. 2017) and grasspea (Wiraguna 2021), and salinity in mung bean (Iqbal et al. 2022 in prep.) Report available on identification of traits responsible for tolerance to waterlogging in mungbean (Khin Lay et al. 2021), pea (Zaman et al. 2019) and grasspea (Wiraguna 2021) and underway for salinity in PhD of Md Iqbal	Screening protocols transferred and used by BARI (and UWA) in germplasm screening in Activity 4.2 Output available for use in selection by national breeding programs
4.2 Screening of pulse germplasm to identify sources of stress tolerance	Reports available on identification of sources of water-logging tolerance of mungbean (Khin Lay 2022), lentil (Wiraguna et al. 2017), pea (Zaman et al. 2019) and grasspea (Wiraguna et al. 2020), and salinity in mung bean (underway for salinity in PhD of Md Iqbal) using protocols from #4.1	Identified sources of stress tolerance in mungbean shared with LWR/2014/73; to be used as parents in crossing by national programs. Phenotyping data used for GWAS to understand genetic control/candidate genes for stress tolerance with WorldVeg/CIM-2014-073.

4.3 Field evaluation of germplasm of salt tolerant fodder options	Report not available on new feed options. Grasspea and field pea identified as viable fodder options.	
New Activity 4.4 UWA completion of experimental work for: • Improved protocol for genetic modification procedures • SOP for clonal propagation of mungbean • SOP for year-round accelerated Single Seed Descent (aSSD)	Reports available on: • Improved protocol for GM procedures • SOP for clonal propagation • SOP for aSSD	Information available for future mungbean improvement for researchers e.g. WorldVeg, International Mungbean Improvement Network (CROP-2019- 44) and Australian national breeding program

7 Key results and discussion

Objective 1: To understand the context for practice change in dry season cropping in Southern Bangladesh, and establish its evolution as a response to the project

Activity 1.1 Focus group discussions (FGD) to understand incentives, requirements and obstacles to dry-season cropping

and Activity 1.3 Assessment of availability & gender dimension of additional labour requirements as options to increase crop productivity and to cut and carry fodder for livestock

Briefly, mungbean and grasspea are two important dry season crops in Barisal Division. Mungbean is planted in January-February and harvested in May. The harvesting and post-harvest processing of both crops is labour intensive (see 1.3 below). Recently, the production of mungbean substantially increased in the districts of Patuakhali and Barguna but has decreased markedly in Jhalakati district. A down-trend in mungbean production districts is also apparent in Bhola and Barisal.

Grasspea is a low input-requiring crop usually relay planted with *aman* rice crop without land tillage in November. Farmers rate grasspea as profitable and a preferred crop compared with other dry season options. There has been a drastic reduction of grasspea production throughout Barisal Division, attributed by farmers to waterlogging or land inundation due to excessive rainfall in the month of November. Analysis of long-term rainfall indicates it is not the culprit. Instead, analysis of long term non-tidal river water level data indicates higher water levels in major rivers in Nov-Dec since 2008. High river water level during lean period might have caused slow land drainage. Waterlogging induced grasspea seedling damage can be attributed to slow drainage coupled with rainfall in November. Development of infrastructure like house construction, roads, etc. in the community also causes barrier to rainwater drainage after heavy showers. As a result of this finding of the importance of water logging on pulse production, an increased project emphasis was made on waterlogging tolerance screening of pulses under Objective 4. The identified decreasing trend in area under grasspea was reflected in reduced project emphasis on grasspea activities balanced by increased activity of cowpea research.

ARF found the shortage of farm labour as a serious problem in the region. Nearly 90% of farming communities comprise small and marginal farmers/households, whose livelihoods rely heavily on both farm and off-farm incomes. As farm labour opportunities are both seasonal and for a short duration, farm labourers are increasingly exiting agriculture to seek employment in non-farm sectors (in urban areas, business centres etc). The labour shortage has led to a rise in wages, discouraging small/ marginal farmers from hiring farm labour. To earn off-farm income and to sustain crop production women in marginal and smallholder farm families are increasingly engaging in agricultural operation as a coping strategy to the labour shortage. This is particularly true for mungbean and grasspea production, harvesting and post-harvest operations. Farmers are receiving a poor return of their mungbean produce due to unavailability of de-husking facilities for the crop at the village level. As a result of this finding a new project emphasis was made on community dehusking mini mills. Mungbean was identified as a woman-friendly crop since most operations are done by women. Gender implications of field pea harvested and marketed for green pods - considered women’s work - changed the projects pea focus to green pea pods as opposed to dry grain use. Societal barriers against the engagement of women in agriculture are being eroded. However, while women’s involvement in agriculture is visible in landless and small farm families, women in well-off families do not yet engage in agriculture.

Activity 1.2 Baseline and endline surveys of households in targeted project region

Briefly, the BAU baseline survey found that farmers in non-saline areas had cropping intensities above 2 crops/yr, whereas their counterparts in saline areas had cropping intensities below 2 and lower productivity and profitability of major crops (Uddin et al., 2019). The opportunity to diversify cropping in non-saline lands contributed to overall improved household livelihoods and the percentage of deprived households was 41.7%. But in saline areas, restricted to cropping just *aman* rice, the percentage of deprived households was greater at 56%. Research for saline areas on options for dry season crop diversification is clearly warranted. In addition to the problem of salinity, saline-area farmers had problems with lack of mechanization and existence of leasing arrangement of water canals.

Another survey conducted using an independent sample was conducted in 2021 to understand the context for practice change in dry season cropping in non-saline areas of Southern Bangladesh, and to compare practices between focal farmers and non-focal farmers in neighbouring villages. It was found that the rate of migration in the study areas had increased in the past years, mainly due to the occurrence of natural disasters, economic and health issues. Pulses covered around 37.0 and 33.0 percent of the total cropped area of focal and non-focal farmers, respectively (Uddin et al., 2021). Nearly 100% of focal farmers used improved crop varieties and the recommended seed density at the end of the study, along with timely application of fertilizers and insecticides by 80% focal farmers. The crop intensification index indicated that the cropping intensity of focal farmers had increased by ~3% after the project intervention, but increased by only 1.6% among non-focal farmers. Average annual income of focal and non-focal farmers was Tk 102,798 and Tk 100,846, respectively at the end-line period, where the contribution of pulses production on the farm income was 45.5 and 43.1 %, respectively. Farmer livelihoods were analysed by the livelihood capital (i.e., human, social, natural, physical and financial capitals) framework - known as sustainable livelihood framework. The livelihood capital of both focal and non-focal farmers had increased at the end-line period. However, the extent of increase was higher for focal farmers, and the proportion of privileged focal and non-focal farm households based on poverty dimensions was 71.0 and 65.2 %, respectively.

The study found the availability of improved crop varieties as the major strength, land transformation from crop land to other enterprises as the major weakness, improvement in farm mechanization as the major opportunity and environmental vulnerability as the major threat of agriculture in non-saline areas of Southern Bangladesh. Recommended points of intervention based on the findings are:

i) selecting the best-suited pulse variety for production in each study area based on soil topography and geographical condition;
ii) direct input provision by the government to support farmers with regular extension services;
iii) training programs and field demonstrations by government and non-government organizations to improve farmer knowledge on product value-addition activities; and
iv) providing farmer access to modern agricultural equipment/machinery, as well as adequate crop storage facilities.

NEW Activity 1.6 Piloting of mini mills to dehusk mungbean in Barisal Division by ARF and PEP

Following the mid-term evaluation of the project, it was decided to pilot mini mills to dehusk mungbean. While small and marginal farm households in Barisal Division produce two-thirds of the country’s mungbean, the farmers receive a poor return of their produce due to the unavailability of de-husking facilities for mungbean at the village level. While rice, wheat, and spice de-husking/grinding machines are available in most villages, women dehusk their mungbean grain with locally-fabricated grinding stones, resulting in high losses of broken or split grain. Following de-husking, women clean them by *kula* (traditional flat object made of bamboo) to prepare them for cooking. As this process is laborious/time consuming, households that produce mungbean consume very little, i.e. weekly/bi-monthly. Currently, farmers usually sell their un-dehusked mungbean grain either to middlemen or in the local market at one-third to half of the market price. Women prefer to buy pulses from the market because milling involves time consuming post-harvest preparation. There is a significant price gap between entire grains of mungbean at the farm gate (Tk 50-60/kg) and de-husked mungbean at the final point of sale (Tk 130 to 170 /kg).

Interviews with rice millers revealed that operating a single-use mini mill (like mungbean mill only) might not be economic, as mungbean grain is available in the market for only 2-3 months a year. From an economic perspective it is necessary to associate a mungbean mill with other existing devices, like rice mill, wheat mill, spices mill, etc. Accordingly, two pilot mini mills were installed by ARF in mungbean growing areas - both as an ancillary to an existing milling business. The first mini mill was in Patuakhali district in Boro Awliapur village, close to a BARI OFRD research hub and Muchihat bazar, at the rice mill of Mr. Shahidul Islam Talukder. The second mini mill was installed at a rice husking mill in Rahutkati, a village market about 6 km from BARI Regional Agricultural Research Station Rahmatpur, Barisal. The location is well connected by road and river communication and the surrounding area is a centre for mungbean production.

Mungbean processing mills are unavailable locally. Considering the sustainability of a de-husking processing system, we gave local machine manufacturers who used locally available machinery and spare parts within the country priority to develop the mini mills. ARF contracted Mr Hemayet Khan, a local fabricator of mungbean dehusking machines to build and install machines and train the operators at the two mills. The machines were made operational by April 2021 despite COVID issues. The manually operated units have a milling capacity of 300-500 kg/8-hr day, run on electricity, and - in addition to mungbean - can also dehusk lentil and chickpea, using the ‘wet’ method.

At Rahutkati, the mill began operating on 13 April 2021. Mohsin has been running the mini mill on farmers mungbeans, but the volume of daily mungbean processing is low, and up to 15 July 2021, 507 kg mungbean and 54 kg lentil were processed.

The third mungbean processing mill was established at ARF Agro-processing center, Savar. ARF opted to install a semi-automatic mungbean dehusking machine imported from China with the co-financing of ARF Agri-Business Program. The imported unit is a ‘Dry Method’ dehusker which arrived on site in May 2021. The machine has an electrical power requirement of 18.5 W at 440 volts. ARF has entered into an agreement to run the machine in collaboration with the Krishibid Group, an agri-business conglomerate in Bangladesh.

Building on the ARF research, in 2021/22 PEP installed a further eight mungbean de-husking facilities at the community level in mungbean growing areas of the districts of Patuakhali (5 mini mills) and Barguna (3). Following the installation of the mini mills, the demand for de-husking mungbean grains was noted to rapidly increase in these communities. As a result, mungbean consumption at family level is increasing. Further mini mills (21) have been recently introduced by PEP during the extension period. There are 10 more for installation.

The main intention of the project is to make sustainable mini mills system at community level/mungbean growers’ level, so that local mungbean farm households may be able to de-husk their mungbean which they may sale in the local market with good price and may be able to consume at their family/community level to improve household nutrition.

Objective 2: To understand and evaluate production technologies for dry season cropping of pulses in saline-free land to increase productivity and profitability in the Barisal Division in Southern Bangladesh

Activity 2.1. Evaluation on-farm of improved mungbean technologies (cultivars & management practices) and Activity 2.5 on Mungbean demonstrations

Adaptive trial of varieties: To evaluate the performance of released mungbean varieties in Barisal Division and decide which to use for on-farm demonstration, a field experiment was conducted during late *rabi* season at three sites in Yrs 1 and 2. Overall, BARI Mung 6 followed by BARI Mung 5&7 were identified as the highest yielding. It was decided that this trio of cultivars can be used for on-farm trials and demonstrations in the Barisal Division. The trial was discontinued.

Effect of sowing date: An on-farm study of sowing date was conducted on farmers' fields at two sites during late *rabi* season of 2018. Sowing dates were from early January until March 1st. The highest yields were achieved with January sowing, tailing off with seeding in February to reach a 50% reduction of January yield by March 1st. Farmers indicate that the fields are rarely available to sow at the beginning of January because of the harvest of *T. aman* rice. The period of 15-30 January appears to give the best performance and is practical for growers and often gives one more pick - delayed sowing into mid-February and later exposes the crop to increasing temperatures and insect attack, and to an increased risk of flash flooding from heavy rainfall events. The repeat sowing date trial in 2019 was damaged by heavy rain. The trial was discontinued, and January sowing was incorporated into the demonstration package.

Winters are becoming warmer in the Coastal belt and early sowing of mungbean has practical de-risking advantages allowing early growth and development in January/February. This avoids in late spring the effects of increasing temperatures, soil salinity build-up, insect attack and the increased risk of flash flooding from heavy pre-monsoon rainfall events.

Effect of sowing method: An experiment to compare sowing methods in mungbean was conducted on-farm in 2018 and 2019. Four sowing methods were compared and averaging over sites, line sowing (both treatments) gave higher yield than either broadcast (T_1 – 1240 kg/ha) or farmers' method (T_4 – 1180 kg/ha). Mechanical sowing not only gave a high yield, but facilitated rapid field coverage at seeding, and latterly also weeding between the rows. With mechanical sowing, percentage of matured pod during first pick is also higher than conventional seeding. Seed drilling was incorporated into the mungbean production package.

Weed control: Weed control remains a problem in mungbean. Weed control options were examined on a farmer's field using line sowing in 2018 and 2019. In summary, over the years, the application of Whip Super™ (Fenoxaprop-p-ethyl) herbicide was not a consistent improvement to hand weeding in mungbean. In Yrs3 and 4 another trial on weed management was conducted in farmers' fields with five treatments: W1= Control; W2= One hand weeding 20 days after emergence (DAE); W3= BARI weeder (hand-operated row hoe) at 20 DAE; W4= W2 + BARI weeder at 40 DAE; W5= Spraying of Quizalofop-p-ethyl (Weednil™) at 20 DAE@ 1.5 ml/l. Averaged over Yrs3 and 4, there were marked increases in yield of more than 50% over the control from weed control with treatments: W3 - BARI weeder at 20 DAE; W4 - One hand weeding at 20 DAE + BARI weeder at 40 DAE; and W5 - Weednil™ (Quizalofop-p-ethyl) application (Table 7.2.1). Clearly, weeds are a major factor limiting crop productivity. Considering the mean over years for benefit/cost ratio, the weed control options rank as first - W3 - BARI weeder at 20 DAE, followed by W5 - Herbicide, and then W4 - One hand weeding at 20 DAE with BARI weeder at 40 DAE. These three weed control treatments offer farmers diverse options for weed control in mungbean.

Table 7.2.1 Seed yield of mungbean and benefit/cost ratio (BCR) in a weed control trial of treatments in farmers' fields at Babuganj, Barisal in 2020 and 2021. Means followed by a different letter in a column are significantly different at P=0.05.

Weed control treatments	Seed yield (kg/ha)			Mean
	2020	2021	Mean	BCR
W_1 - Control	966 c	747 c	857	2.93
W_2 - One hand weeding at 20 DAE	1147 bc	1060 b	1104	3.01
W_3 - BARI weeder at 20 DAE	1332 ab	1477 a	1404	3.71
W_4 - W2 + BARI weeder at 40 DAE	1539 a	1543 a	1541	2.75
W_5 - Weednil^{TM} herbicide @ 1.5 ml/L at 20 DAE	1504 a	1197 b	1350	3.10
LSD $_{(0.05)}$	116.8	240.5		
CV (%)	4.6	10.6		

Integrated pest management (IPM): Insect pests particularly thrips and pod borers are major yield reducers in mungbean. The effectiveness of IPM approaches using botanicals, synthetic insecticides and blue sticky traps was evaluated at RARS Rahmatpur, Barisal during late *rabi* 2018 and 2019. Thrip infestation in mungbean flowers was reduced by the installation of blue sticky traps (from the World Vegetable Centre) plus two applications of azadiractin (Bioneem plus 1EC) at flower initiation stage and peak flowering stage. In 2019 considering environment friendliness, the installation of blue sticky trap & pheromone trap + two applications of azadiractin (Bioneem plus 1EC) @ 1 ml/l + third spraying with spinosad (Success 2.5 EC) @ 1.2 ml/l was the best IPM package to control major insects with higher yield of mungbean. But the cost of the treatment was greater than farmer insect control by Imidacloprid. In Yrs3 & 4 another approach to pest management was taken with micro and macro-nutrients in trials at Ishurdi and Rahmatpur (Yr3 only). In 2020 the application of Sulphur (Thiovit 80 WG) significantly reduced thrip flower infestation and pod infestation by borers compared to the control. The overall yield advantage of Sulphur application over control was 13% and its marginal benefit cost ratio was 2.79 (Table 7.2.2). This was greater than the application of some other nutrients which, although they yielded more, were more costly.

Table 7.2.2. Effect of foliar application of micro and macro-nutrients on seed yield and marginal benefit/cost ratio (over control) of mungbean in 2020 and 2021. All means followed by same letters at each column were not significantly different by LSD All-Pairwise Comparisons Test at 5% level of significance.

Treatments	Seed yield (kg/ha)			Mean
	2020	2021	Mean	MBCR
T1 = Untreated control	1043 a	1480 c	1262	-
T_2 = Microvit 80 WP @ 1 g/l	1100 a	1707 ab	1404	1.92
T_3 = Thiovit 80 WG @ 2 g/l	1135 a	1710 ab	1422	2.79
T_4 = Nutra-phos-24 @ 3 g/l	1169 a	1865 a	1517	2.42
T_5 = McChili @ 0.35 g/l + Solubor @ 1 g/l	1120 a	1712 ab	1416	1.91
T_6 = Muriate of potash @ 10 g/l	1080 a	1591 bc	1336	1.48
	NS	*		

Demonstrations - Productivity and profitability

In 2021 (Yr4) the average yield of mungbean demonstrations was 1.41 t/ha compared to 1.12 t/ha in neighbouring farmer-tended plots, indicating a yield advantage of 26% (Table 7.2.3). The trend was like that of the previous three years of demonstrations: In Yr1 the corresponding data were mean yield of demonstrations at 1372 kg/ha compared to 954 kg/ha in neighbouring plots. In Yr2 the average yield of demonstrations was 1144 kg/ha compared to 906 kg/ha in neighbouring farmer-tended plots, while in Yr3 the average yield of demonstrations was 1280 kg/ha compared to 1070 kg/ha in neighbouring farmer-tended plots. Over years, the average yield gap between demonstration package and adjacent farmer plots was 289 kg/ha indicating a yield advantage of 29%.

Over the demonstrations conducted in 2021 across Barguna, Barisal, Bhola, Jhalokati, Khulna, Patuakhali and Pirojpur, the average gross margin was 47,983 Tk/ha with a mean benefit-cost ratio (BCR) of 2.62 - using a unit price of 60 Tk/kg of mungbean grain. The BCR was highest in Patuakhali (3.06) and lowest in Bhola (2.23) where only small areas of the crop are normally grown. In the previous year the BCR was again highest in Patuakhali (1.79) and lowest in Khulna (0.91) where the crop is not normally grown.

The ARF survey indicated that increasing involvement of women in mungbean production in the community has increased their mobility in the community which helps not only to increase the income of women but also their empowerment at household and community levels.

Table 7.2.3. Mungbean demonstrations in coastal Bangladesh from 2018 to 2021. Gross margins in 2019 and 2020 are based on a price of Tk 50 /kg mungbean and Tk 60 /kg in 2021.

	Year				
	2018	2019	2020	2021	Mean
Demonstration yield (t/ha)	1.37	1.14	1.28	1.41	1.30
Farmer control (t/ha)	0.95	0.91	1.07	1.12	1.01
Yield gap (kg/ha)	418	238	210	290	289
Advantage over control (%)	43.8	26.3	19.6	25.9	29
No. demos	14	12	7	7	
Gross margin (Tk/ha)	na	30,969	35,823	47,983	
Benefit/cost ratio (BCR)		2.12	1.23	2.62	

na - Not available.

Seed production (also relates to Activities 2.2 - 2.5): Breeder seed production of grasspea, field pea, cowpea and mungbean was undertaken by PRC at different BARI stations during *rabi* and *kharif* seasons each project season for use in subsequent demonstrations and trials. Over the duration of the project from 2018-2021, a total of ~9.4 t seed of different pulses (Cowpea 345 kg, Field pea 3.1 t, Grasspea 3.6 t and Mungbean 2.3 t) was produced (Table 7.2.4).

Table 7.2.4. Pulse seed production (kg) for the project by crop and harvest year.

Crop	2018	2019	2020	2021	Total
Mungbean	255	664	497	925	2341
Grasspea	500	1,045	1,015	1050	3610
Cowpea	150	100	20	75	345
Field pea	600	900	1,085	520	3105
Total	1505	2709	2617	2570	9401

Activity 2.2. Cultivar identification and evaluation on-farm of improved cowpea technologies and Activity 2.5 demonstrations on cowpea

Germplasm evaluation: During late *rabi* seasons of Yrs 1 to 4, eight selected cowpea lines and a local check variety (BARI Felon-1) were assessed at RARS Rahmatpur, Barisal. The line CPL-8-17 has dramatically out-yielded the local check in Yrs 1-3. In 2021 the trial was repeated at three sites CPL-8-17 again out-yielded the local, but the difference was non-significant. In Yr4 differences among entries for seed yield were non-significant. Overall, CPL-8-17 was consistently out-performing the local control and is clearly a very strong candidate for release and rapid seed multiplication. In 2023 one advanced cowpea line will be submitted to the National Seed Board for varietal release, and next year in 2024 it is planned that another high yielding and early line among the second tranche of germplasm will also be released.

Production practices: Existing best practices for cowpea production were evaluated on-farm in the coastal region in the 2017/18 and 2018/19 seasons. Following discussion during the MTR/annual planning meeting, the on-farm evaluation of cowpea practices was terminated, and in the 2019/20 season the production package was transferred into project demonstrations (Activity 2.5).

In Yrs 2-4 cowpea mulching was evaluated in order to retain moisture in the soil through reducing evaporation and hence the increase in soil salinity in winter cowpea production. The aim was to compare different mulch materials (no mulch, rice straw. polyethylene and rice husk). Mulching increased seed yield significantly by decreasing soil salinity and conserving soil moisture compared to no mulch. The highest benefit/cost ratio (BCR) (1.49) was from straw mulch indicating its suitability for cowpea cultivation regarding productivity and economic net return in saline areas.

Demonstrations:

Cowpea is a traditional crop of the coastal region that is usually cultivated relaying with T. *aman* rice. Sowing methodology and time are like grasspea. In the last three years another sowing method, drilling by seeder, was practiced in some locations of Patuakhali and Barguna district.

Block demonstrations of cowpea were conducted in the *rabi* season sown from Yrs1-4 in Barguna, Bhola and Patuakhali. The technology package comprised line sowing by PTOS of cultivar (BARI Felon 1) with fertilizer (NPKS 20-40-20-10 kg/ha). Insecticide was applied to control pod borer, as necessary. The results show that average yield ranged over seasons from 1.27-1.60 t/ha with a mean of 1.4 t/ha (Table 7.2.5). Farmer control yield averaged 0.93 t/ha giving a mean yield gap of 460 kg/ha and a yield advantage of the package of 49%. The benefit-cost ratio (BCR) for cowpea production was each year lower in Bhola than Patuakhali. For example, in 2021 the BCR was 2.1 in Bhola and 3.27 Patuakhali. The substantial yield gap and the cost-benefit ratio in Patuakhali should encourage cowpea uptake as a dry-season cropping option.

Table 7.2.5. Results of cowpea demonstrations in the rabi season from 2018-2021.

	Year				
	2018	2019	2020	2021	Mean
Demonstration yield (t/ha)	1.28	1.6	1.4	1.27	1.39
Farmer control (t/ha)	0.88	0.94	0.91	0.98	0.93
Yield gap (kg/ha)	394	665	490	290	460
Advantage over control (%)	45	71	54	29	
No. demos	4	5	3	4	
Benefit/cost ratio		2.24	0.6 in Bhola to 1.44 Patuakhali	2.1 in Bhola to 3.27 Patuakhali	

In the coastal belt of Bangladesh, project research shows cowpea has much higher potential than at present. The four seasons of block demonstrations of relay production package showed consistent yields from 1.3 to 1.6 t cowpea/ha with a mean yield advantage over adjacent farmer plots of 460 kg/ha. The benefit-cost ratio (BCR) for cowpea production was always highest in Patuakhali/Barguna, where the crop has greatest scope. Furthermore, the relay-sowing package was with the local cultivar BARI Felon 1, and our germplasm evaluation showed that lines such as CPL-8-17 have consistently outyielded the local control. The release of such material as cultivars will further boost the crop’s potential especially in Patuakhali/Barguna.

Activity 2.3. Cultivar identification and evaluation on-farm of improved grasspea technologies and Activity 2.5 demonstrations on grasspea

Traditionally farmers in the southern belt grow grasspea as a relay with T. *aman* rice, but they only achieve low yields due to the use of the local variety and poor crop management. In December 2017 the southern belt of Bangladesh experienced unseasonal heavy rains that caused extensive flooding. Pulses sown prior to this weather event experienced severe water-logging and our experimental program of grasspea was severely damaged.

Germplasm evaluation of eight selected lines from ICARDA was conducted from Yrs1 to 4. The trial at RARS, Rahmatpur, Barisal was exposed to severe waterlogging in December 2017. Lines IGYT-125 and BARI Khesari 3 were more tolerant to waterlogging at the seedling stage than other entries and line IGYT 216 survived the flooding - as seen in the travelling workshop - to give the highest grain yield (4.0 g/plant). Over the years IGYT-216 and IGYT-125 out-yielded (albeit often non-significantly) the check BARI Khesari 3. Although the yield advantage over the local check was less spectacular than in cowpea, these lines are to consider for release.

Grasspea production practices: An on-farm trial was conducted to evaluate improved management practices for the relay sowing of grasspea with T. *aman* rice at two locations Barisal and Jhalokathi districts during rabi season of 2017-18. The production package comprised improved variety (BARI Khesari 3), fertilizer application (NPKS at 20-40-20-10 kg/ha with nitrogen top dressing), seed rate of 75 kg/ha and 30 cm stubble height retention of T. aman rice on 1 ha at each location. The rain in December 2017 destroyed the Jhalokathi trial and the lower lying sector of the Barisal site. The remaining upland (0.3 ha) area gave a mean seed yield of 1404 kg/ha. Repeating the trial in the 2018/19 season, the mean plant population was 58 plants/m^2 and the seed yield averaged 1251 kg/ha. Following discussion during the MTR/2019 annual planning meeting, in the 2019/20 season on-farm evaluation of grasspea practices was not undertaken and the production package was used in project demonstrations (Activity 2.5).

Grasspea demonstrations:

The crop is traditional in the southern region with relaying in November as the usual way for its cultivation. In Yr1 *rabi* season a total of 35 grasspea demonstrations were damaged by the December 2017 rains**.** One site in Porijpur was relatively unscathed, where the demonstration yield was 1.3 t/ha compared to the adjacent farmer plot which gave 0.99 t/ha. In the 2020 season a total of ~45 ha of demonstrations of an improved production package were conducted in Barisal, Bhola, Barguna, Patuakhali and Pirojpur. In 2019/20 the yield of demonstrations with BARI Khesari-2 was 1.47 t/ha giving a yield advantage over neighbouring farmers plots of 41%, while the yield of plots with BARI Khesari-3 was 1.42 t/ha with a 67% yield advantage over adjacent farmer fields.

In the 2020/21 season a total of ~25 ha of demonstrations of an improved production package were conducted in Barisal, Bhola, Barguna, Jhalokati, Patuakhali and Pirojpur.

Over the four project seasons it was observed that the yield of pilot production plots of grasspea was 25-60% higher than farmers’ own local variety. Changing variety and the introduction of fertilizer use may be the reasons of the yield increase. In 2020/21, for example, the benefit-cost ratio for relayed grasspea was more than 2.5 in Bhola and Patuakhali but only 1.6 in Barisal. Clearly, the high BCR of relay-sown grasspea shows it as profitable in some areas. But the crop is risky in the coastal region as establishment depends on unusual rainfall and/or flooding in December during germination and early vegetative growth. The crop’s risks were confirmed by the socio-economic survey.

For example, the southern belt of Bangladesh experienced unseasonal heavy rains (35 mm) on 9/10 December 2017 that caused extensive flooding. Pulses including grasspea sown prior to this weather event experienced severe water-logging and our experimental program and farmers' crops of grasspea, field pea and lentil were severely damaged as a result. To assess the probability of such an extreme rainfall event we studied long-term monthly weather data (69 years) for Barisal. Calculating the probability of receiving 40 mm or more in a month, the Figure (7.2.1) shows that the probability of such an extreme rainfall event as that of December 2017 is P= 0.07.

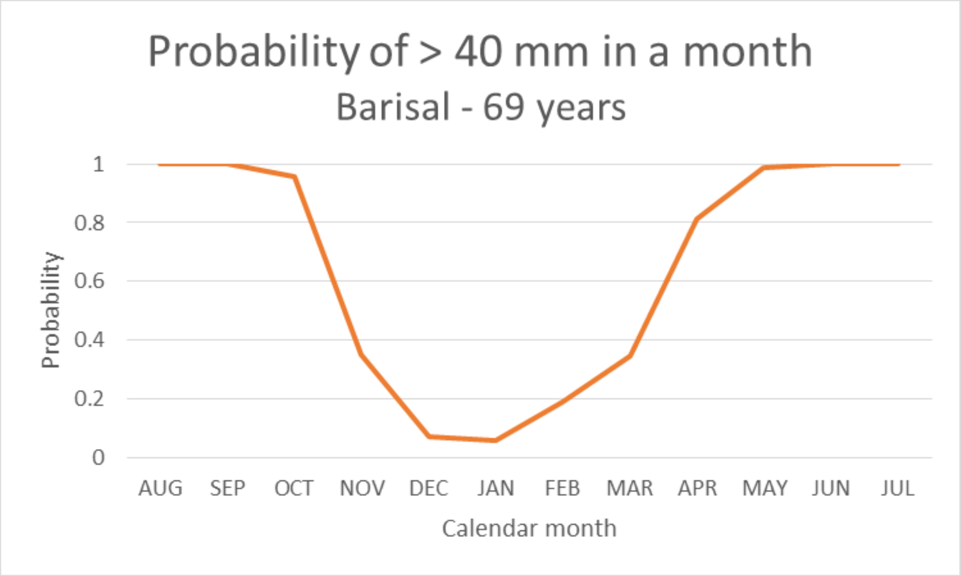

Figure 7.2.1. Probability of more than 40 mm total rainfall in a month at Barisal based on 69 years data.

Activity 2.4: Evaluation on-farm of relay-sowing of lentil and *green pea and Activity 2.5 of demonstrations on lentil and pea*

Lentil production technology: A package for lentil production using relay sowing was produced for Western Bangladesh in the previous project (CIM-2009-038). Although lentil is not widely grown in Southern Bangladesh it has great potential on well drained land in the Barisal region - especially charland (river sands). A field trial was conducted on relay-sown improved management practices of lentil in char-land (actively eroding/accreting riverine deposits) in Barisal in Yr1, but the trial was washed out by the rains of early December 2017. In the following two *rabi* seasons an on-farm trial was conducted with the lentil relay sowing package at three villages in Barisal. The mean seed yield of lentil was 1252 in 2019 and 1457 kg/ha in 2020. These results were promising, and the practices were integrated into project demonstrations (Activity 2.5).

Demonstrations of lentil

Lentil demonstrations of an improved production package were undertaken from Yrs1 to 4 on upland soils in Barisal and Bhola. A total of 16 demonstrations of lentil were abandoned due to damage from heavy rainfall in December 2017. In each of the following three seasons lentils were demonstrated with an overall mean yield of 1.4 t/ha (Table 7.2.6). As lentil is uncommon in Southern Bangladesh it was difficult to find neighbouring fields for comparative purposes. The gross margins varied from 53,500 to 38,800 Tk/ha with a unit lentil price of 50 Tk/kg. Lentil as a crop has promise in well-drained pockets of land (e.g., charland - river sand) in Barisal and especially in Bhola where there is extensive charland.

Table 7.2.6. Lentil demonstrations from 2019-2021.

	Year			
	2019	2020	2021	Mean
Demonstration yield (t/ha)	1.45	1.42	1.4	1.42
Farmer control (t/ha)	na	1.05	0.87	0.96

Yield gap (kg/ha)		370	530	450
Advantage over control (%)		35	61	
No. demos	1	1	1	
Gross margin (Tk/ha)	38800	37300	3550	
BCR	2.15	0.9	2.06	

* na - Not available; ** using a unit price of 50 Tk/kg for lentil grain

Pea production technology: Relay sowing of field pea is practiced in Western Bangladesh for green pod production, but the crop is not grown in the southern belt. This on-farm study was undertaken to evaluate pea as a relay crop with T. *aman* rice in Babuganj, Barisal for green pod production during *rabi* season of Yr1, but unseasonal rains in December 2017 destroyed most of the low-lying crop, the surviving 0.2 ha gave a mean grain yield of 963 kg/ha. In the following *rabi* season the mean green pod yield from the package was 7.5 t/ha. These results look promising, and the package was moved into project demonstrations.

Demonstrations of pea

As with lentil, pea is not widely grown in Southern Bangladesh. Demonstrations of pea were conducted each season from Yrs1 to 4 in plots in Barisal (Table 7.2.7). In Yr1 as in other *rabi* pulses the 8 pea demonstrations were abandoned due to damage from heavy rainfall in December 2017. In following season, the plot yield was 0.8 t grain/ha with a gross margin was 8,500 Tk/ha giving a benefit-cost ratio of 1.36 using a unit price of 50 Tk/kg of dry pea grain. In 2020 the grain yields were 1.65 t/ha in Barisal and 2.8 t/ha in Bhola. In 2021 the grain yields were low in Khulna (0.34 t/ha) but averaged 1.28 t/ha overall. Omitting Khulna, the mean gross margin was 41,948 Tk/ha while the benefit/cost ratio averaged 2.86. The highest BCR (3.71) was in Patuakhali.

Pea growing households indicated that it’s a new crop in the region with gender implications. Field pea for green pods taken as relay in *aman* rice field is considered as a low input cost technology and is easy to handle by women-headed households. Green pods of pea can be harvested weekly by women in the household, and, with a very good demand in the local market where farmers are selling around Tk 40-50/kg, the technology is encouraging for households to adopt. With no apparent pest/disease infestation during the crop life cycle of the crops, thus with no requirement for agrochemicals green peas can be consumed raw without further processing. After harvesting of field pea, mungbean can be cultivated in same land, so there is scope for further cropping intensification. The crop has particular promise as a green vegetable, rather than as a grain, after completion of the Padma bridge. In the last two seasons farmers are very happy to see the outcomes of relay green pea in *aman* (monsoon) rice crops introduced by the CIM-2014-076 project. They are making reasonable profit using very minimum production cost. This additional crop is also helping to improve children as well as the rest of the family’s nutrition as most children like it as green pea. The variety BARI Motor 3 is dual purpose - green pod and dry seeds.

Table 7.2.7. Pea demonstration production and benefit cost ratio (BCR) from 2019-2021.

	Year			
	2019	2020	2021	Mean
Demonstration yield (t/ha)	1.45	1.42	1.4	1.42
Farmer control (t/ha)	na	1.05	0.87	0.96
Yield gap (kg/ha)		370	530	450
Advantage over control (%)		35	61	
No. demos	1	1	1	
Gross margin (Tk/ha)	38800	37300	3550	
BCR	2.15	0.9	2.06	

* na - Not available; ** using a unit price of 50 Tk/kg for pea grain

Objective 3: To identify wheat germplasm with salinity tolerance adapted to Southern Bangladesh.

Activity 3.1. Establishment of set of lines and their evaluation to benchmark current and future wheat germplasm in two polder sites with LWR/2014/073

A benchmark set of 24 wheat lines was established consisting of 12 wheat varieties previously and/or currently grown in Bangladesh (BARI Gom varieties), 9 advanced breeding lines from BWMRI (BAW lines) and 3 putatively salt-tolerant wheat varieties (KRL lines) from India.

The benchmark set of wheat lines was evaluated at a total of 16 field trials at 8 locations over two years in southern Bangladesh. The most useful field data came from the 2017-2018 season due to a broad range in salinity levels across the 10 trial locations that year. However, data from three of the 10 trial sites were not used due to very low heritability for yield at these sites. Salinity levels increased significantly from sowing to anthesis at six of the seven field trial sites, with the exception of the trial at Barisal which remained unchanged with very low salinity levels. The small range in salinity levels measured at all trial sites at sowing was indicative of a much larger range of higher salinity levels measured at anthesis. There was a clear negative linear relationship between salinity levels measured at each site (ECe) and site mean grain yield across the seven field trial locations. This relationship allowed for the ranking of salt tolerance of all 24 benchmark wheat lines to be calculated on the basis of the slope of the yield response across the seven field sites used in the 2017-2018 season, a more negative slope indicating a greater yield decline due to increasing salinity (see Figure 7.3.1 for contrasting examples of salt tolerance). Ranking genotypes for salt tolerance using this approach factors in all the yield responses from moderate to high salinity sites and importantly includes any genotypic variation in yield at the higher yielding low salinity locations as a baseline for the response.

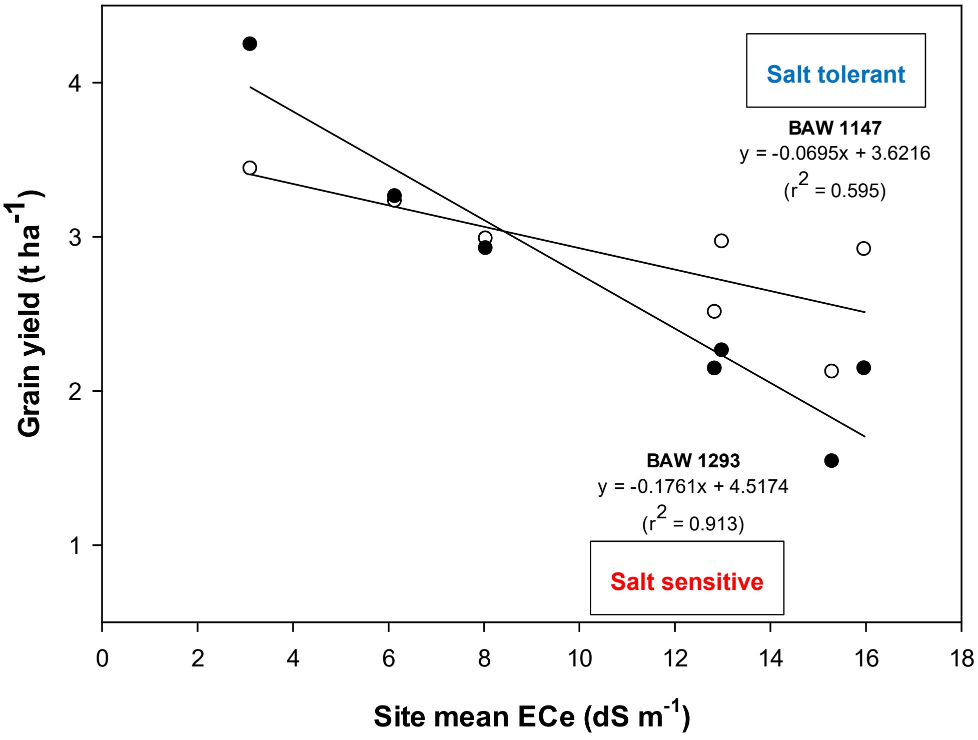

Figure 7.3.1. Relationship between salinity (site mean ECe,) at anthesis and grain yield of two contrasting genotypes at seven field trial locations in southern Bangladesh for the 2017-2018 field season.

Two (of the three) putatively salt tolerant KRL lines (KRL 19, KRL 1-4) together with BARI Gom 25 (with a salt tolerance reputation) ranked in the top four most salt tolerant lines, the results of which appear to validate this approach (Table 7.3.1). For comparison, genotypes were also ranked based on average yield at the three lowest yielding (high salinity) sites (Table 7.3.1). There was a poor correlation between salt tolerance rankings using both methods ($r^2 = 0.092$, n.s.). However, of note is the performance of advanced breeding line - BAW 1147, which ranked 2 for highest yield response to salinity, and ranked 1 for the highest average yield on high salinity sites. Similarly, BARI Gom 25, also ranked highly for salt tolerance using both methods (Table 7.3.1). What needs to be targeted in a breeding program and for eventual release are salt tolerant wheat lines that are also high yielding (i.e., well adapted) on both saline and non-saline field environments.

Table 7.3.1. Salt tolerance ranking of 24 benchmark wheat genotypes based on (a) the slope of yield response across seven sites in south Bangladesh over the 2017 – 2018 field season (see Figure 3.1), (b) the mean yield of the three lowest yielding (high salinity) sites from the same year.

Rank	**Genotype**	**Slope**	**r^2**	Three lowest yielding sites	
				Mean yield (t ha^{-1})	**Rank**
1	KRL 19	-0.0476	0.246	2.063	8
2	BAW 1147	-0.0695	0.595	2.534	1
3	KRL 1-4	-0.0739	0.345	1.763	22
4	BARI Gom 25	-0.0768	0.381	2.087	6
5	BARI Gom 23	-0.1001	0.657	1.924	17
6	BARI Gom 30	-0.1005	0.528	2.226	2
7	BAW 1208	-0.1070	0.742	2.128	4
8	BAW 1272	-0.1088	0.555	1.996	13
9	BAW 1280	-0.1096	0.830	2.011	10
10	BAW 1286	-0.1122	0.654	1.843	20
11	BARI Gom 29	-0.1142	0.764	2.097	5
12	BARI Gom 32	-0.1177	0.818	1.932	16
13	BARI Gom 27	-0.1241	0.699	1.909	18
14	BARI Gom 33	-0.1295	0.854	1.828	21
15	KRL 210	-0.1319	0.689	1.711	23
16	BARI Gom 26	-0.1324	0.836	2.007	11
17	BAW 1290	-0.1357	0.581	2.064	7
18	BAW 1194	-0.1435	0.638	2.017	9
19	BARI Gom 24	-0.1471	0.939	2.152	3
20	Shatabdi	-0.1491	0.952	1.965	15
21	BAW 1295	-0.1501	0.683	1.528	24
22	BARI Gom 28	-0.1740	0.758	1.996	12
23	BAW 1293	-0.1761	0.913	1.982	14
24	BARI Gom 31	-0.1771	0.831	1.892	19

The benchmark set of 24 wheat lines was also screened for two key salt tolerance traits; (i) Na^+ exclusion and (ii) osmotic stress tolerance (for summary of results, see Activity 3.5). There were no clear relationships between genotypic variation in ranking for either salt tolerance trait and ranking of salt tolerance based on grain yield across a range of saline field environments. The main reasons for this are firstly, a lack of genotypic variation in both Na^+ exclusion and osmotic stress tolerance among most of the 24 benchmark lines and secondly, yield being ‘confounded’ by variation in other adaptive agronomic features such phenology, height, disease resistance, etc.

Activity 3.2 Screening protocols and equipment established at BARI Gazipur

Supported hydroponics infrastructure used for screening wheat and other species for salt tolerance traits together with a comprehensive Methods manual were developed by the CSIRO team, sent to BARI and then assembled and tested by the BARI Plant Physiology Team. This screening infrastructure and methodology - coupled with knowledge of salt tolerance traits and how to accurately screen for these traits from the July 2018 Experimental Methods workshop - was subsequently utilised by BARI Plant Physiology team to screen a diverse collection of 150 BARI wheat lines for salt tolerance traits (see Activity 3.3).

Trial design and soil characterisation principles and practices for running field trials in salt-affected soils, which were covered in the Experimental Methods Training Workshop, were adopted in the establishment and completion of numerous field trials over five seasons in southern Bangladesh by the BWMRI team (Activities 3.1 & 3.4).

Activity 3.3 Germplasm screening for salinity tolerance traits

A series of supported-hydroponics salt tolerance screening experiments evaluating a collection of 150 BARI wheat lines, was completed by the Plant Physiology team at BARI between November 2019 and March 2020. Plants were grown in 150 mM NaCl for three weeks for the purpose of evaluating Na^+ exclusion capacity and leaf K^+ concentration. While the ion analysis is yet to be completed, a range of growth parameters (plant FW, shoot height and DW, root length and DW) were assessed and analysed. Shoot biomass was reduced by on average about 40% with significant genotypic variation in the growth response to salinity evident. Subsequent Venn Diagram analysis of the various growth parameters resulted in the identification of 26 wheat lines with putatively greater salt tolerance. Some caution needs to be apportioned to these findings as seed size and appropriate controls were not factored into the experimental protocols. The key objective of these screening experiments, being the Na^+ and K^+ analysis of leaf material is still outstanding and when completed, should be analysed in relation to the assembled growth data.

Activity 3.4 Introgression of improved salinity tolerance into locally adapted wheats

The wheat breeding component for this project focused on incorporating CSIRO's salt tolerance *Nax* genes into adapted Bangladeshi wheat varieties for evaluation and validation. A full report of this component of the project can be found in James et al., (2022a). The crossing program using marker assisted selection (MAS), largely completed by the BARI Biotechnology team (led by Dr Yousuf Akhond), culminated in the development of 72 BC_3F_5 advanced breeding lines (containing the *Nax* genes) in BARI Gom 25 and BARI Gom 26 backgrounds. A large validation Na^+ exclusion phenotypic screen of these lines using supported hydroponics (completed at CSIRO) together with a repeated genotypic screen using optimised markers (BARI Biotechnology), allowed for the identification and subsequent confirmation of a number of homozygous (fixed) lines for *Nax1* and *Nax2* in both backgrounds. This data together with previously collected additional agronomic and phenotypic data on these lines provided the basis for the selection of 12 fixed *Nax* lines for comprehensive field evaluation, comprising three fixed *Nax1* lines and three fixed *Nax2* lines in both BARI Gom 25 and BARI Gom 26 wheat backgrounds.

While both *Nax* genes decreased leaf Na^+ concentration, there was a differential response in the phenotypic expression of *Nax* genes to reduce leaf Na^+ levels between the two local varieties selected as recurrent parents. The average reduction in leaf Na^+ concentrations from BC_3 lines in the BARI Gom 25 background was about twice that of BC_3 lines with the BARI Gom 26 background (Figure 7.3.4).

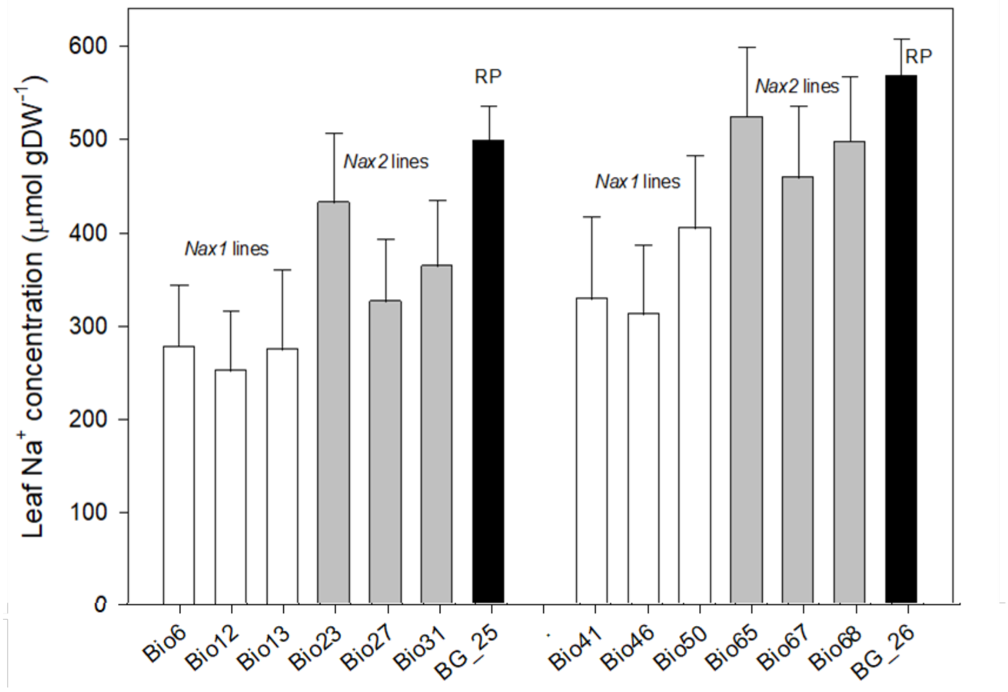

Figure 7.3.4 Leaf Na^+ concentration of *Nax1* and *Nax2* $BC_3F_{3:6}$ fixed lines in two Bangladesh wheat cultivars – BARI Gom 25 (BG_25) and BARI Gom 26 (BG_26) grown in 150 mM NaCl for 10 d. Values are predicted means (BLUES) + S.E. (n=6). RP – recurrent parent.

A series of field trials to evaluate the salt tolerance of these fixed *Nax* breeding lines in BARI Gom 25 & 26 backgrounds was completed at a total of nine locations over three field seasons (2019 – 2022) in southern Bangladesh. Twenty wheat genotypes in total were evaluated including 12 *Nax1* or *Nax2* advanced breeding lines together with their respective recurrent (background) parents. The spatial distribution and range of soil salinity was characterized at all field trial sites using a portable dual EM meter and validated by measuring the electrical conductivity of soil from cores taken from the same locations. These measurements demonstrated the highly variable distribution and severity of salinity even over small areas within field trials, and therefore the importance of tailored spatial trial designs and soil characterisation to account for variable salinity levels in the analysis of yield data. At key sites with moderate to high salinity levels, there was significant negative relationships between individual plot grain yield and ECa measured on the same plots at anthesis.

Table 7.3.4. Average grain yield of BC_3 *Nax* (Bio) lines as a percentage of respective recurrent parents at three low salinity sites and five moderate – high salinity sites, used over three field seasons in southern Bangladesh.

Recurrent parent	Nax status	Line	Average grain yield (% recurrent parent) + s.e.	
			Low salinity sites (n=3)	Mod – high salinity sites (n = 5)
BARI Gom 25	+ *Nax1*	Bio6	87 + 4	119 + 17
		Bio12	88 + 7	122 + 13
		Bio13	83 + 6	123 + 13
	+ *Nax2*	Bio23	81 + 5	103 + 10
		Bio27	71 + 3	112 + 14
		Bio31	91 + 4	117 + 9
BARI Gom 26	+ *Nax1*	Bio41	100 + 3	98 + 6
		Bio46	101 + 3	96 + 5
		Bio50	108 + 8	102 + 7
	+ *Nax2*	Bio65	105 + 13	96 + 6
		Bio67	102 + 7	93 + 5
		Bio68	102 + 8	99 + 4

Table 7.3.4 shows the average yield of *Nax1* and *Nax2* BC_3 lines relative to their recurrent parent at a group of three low salinity sites and also at a group of five moderate to high salinity sites. The key trend of note is that all *Nax1* and *Nax2* BC_3 lines in the BARI Gom 25 background yielded higher on average than their recurrent parent at sites that were characterised with moderate to high salinity levels. In contrast, all *Nax1* and *Nax2* lines in the BARI Gom 26 background yielded similarly to their recurrent parent irrespective of the salinity status of the field trial sites.

This research has confirmed that a targeted trait-based breeding approach can be an effective strategy to improve salt tolerance and subsequent yields of wheat grown in challenging saline environments and confirmed the importance of a thorough field evaluation in targeted environments together with comprehensive site and soil characterization to establish proof of trait value. The key outcome from this comprehensive study was compelling evidence that both *Nax* genes have the capacity to lower leaf Na^+ concentration in Bangladeshi wheat and consequently deliver improved yields in challenging field environments with moderate to high salinity in the southern coastal zone of Bangladesh. The next steps will be crossing the most successful *Nax1* and *Nax2* lines in the BARI Gom 25 background into a range of the most promising locally adapted advanced breeding lines in terms of yield, quality and disease resistance.

Activity 3.5. Screening of wheat lines for salinity tolerance traits at CSIRO (Australia)

To identify wheat germplasm with salinity tolerance adapted to Southern Bangladesh, screening two important wheat collections for salt tolerance traits was completed by CSIRO in Australia:

- ‘Benchmark set’ of 24 wheat lines
- 150 diverse BARI (BWMRI) wheat lines

‘Benchmark set’ of 24 wheat lines

The ‘benchmark set’ of 24 wheat lines was screened for two key salt tolerance traits: (i) leaf Na^+ exclusion and leaf K^+ concentrations, and (ii) Osmotic stress tolerance.

(i) Na^+ exclusion

Significant 2.5-fold variation in leaf Na^+ concentration was found amongst the 24 wheat genotypes, ranging from 245 – 603 µmol gDW^{-1}, with two standout lines (BARI Gom 27, BAW 1290) containing significantly lower Na^+ concentration than the remainder of the wheat lines. While there was a significant range in Na^+ concentration across the set of benchmark wheat genotypes, most genotypes (about 75%) were not significantly different in their capacity to exclude Na^+. There was relatively small variation (about 1.5-fold) in K^+ concentration which surprisingly, was unrelated to Na^+ concentration.

(ii) Osmotic stress tolerance

Screening for Osmotic tress tolerance was completed using an automated imaging platform to non-destructively estimate leaf area and growth rates of wheat lines grown in saline and control conditions. Osmotic stress tolerance was assessed via two different methods; (i) ratio of relative growth rates (RGR) of salt treated versus control seedlings, and (ii) ratio of shoot biomass of salt treated versus control seedlings at the conclusion of the experiment.

Osmotic stress tolerance was assessed as the ratio of the RGR of wheat seedlings grown in a salt treatment versus control at two time intervals of 6 – 13 days and 13 – 16 days after the commencement of the salt treatment. The salt treatment resulted in a significant reduction in RGR relative to controls of all lines on average by 40% at the first time interval, which further decreased to 47% over the second time interval. There was a general consistency in the RGRs of wheat lines under both control and salt-treatment conditions for the first time interval, which resulted in most wheat lines falling into a relatively small range in osmotic stress tolerance of between 0.5 – 0.7. (Figure 7.3.5a).

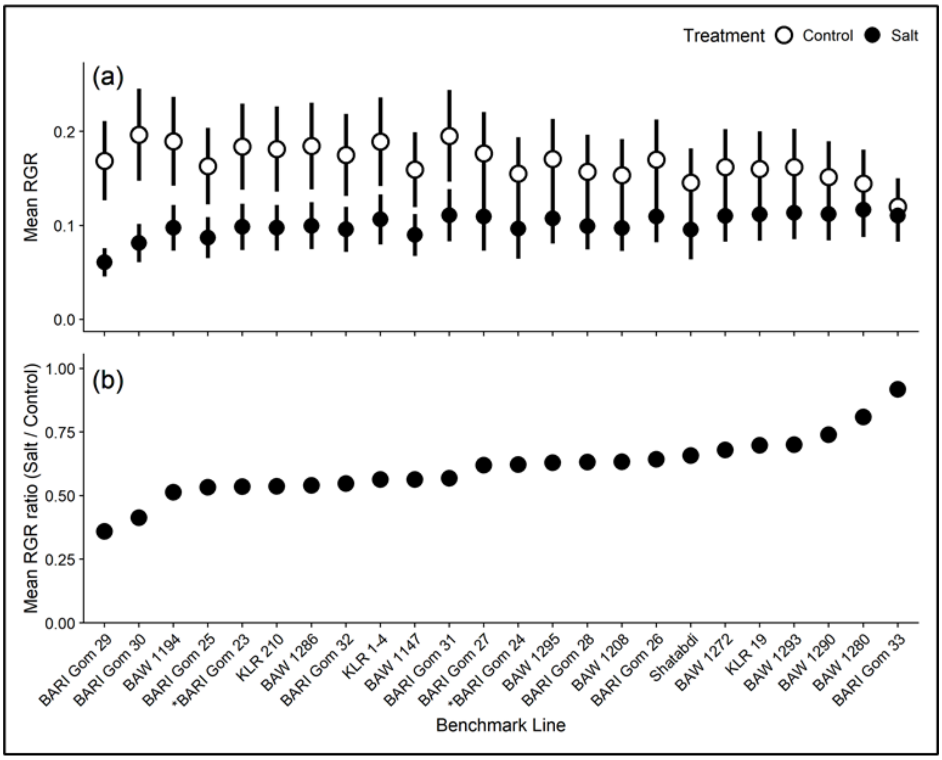

Figure 7.3.5a Osmotic stress tolerance of the ‘benchmark set’ of wheat lines based on the ratio of relative growth rates - RGR (salt / control) for the time interval of 6 – 13 d in 150 mM NaCl.

BARI Gom 33 and BAW 1280 ranked highest in osmotic stress tolerance in this first time interval. The range for osmotic stress tolerance using the ratio of RGR calculated in the second time interval (13-16 d) expanded to 0.38 – 0.74, with BAW 1194, BARI Gom 29 and BARI Gom 33 (again) ranking highest for osmotic stress tolerance using this parameter.

Analysed shoot biomass data (Part ii) showed an average growth reduction due to salinity of 55% with statistically significant genotypic variation in this parameter ranging between 41 – 67% growth reduction due to salinity. While there was a little genotypic variation, most wheat genotypes evaluated (19 of the 24) were not statistically significantly different for this salt tolerance trait. Lines which ranked highest for osmotic stress tolerance using this parameter were BARI GOM 33, BAW 1280 and BARI Gom 25.

There was moderate yet significant positive relationships between osmotic stress tolerance based on the RGR ratio (imaging salt-treated versus control plants) compared to the ratio (salt-treated / control) of shoot dry weights at harvest. This highlights the potential to screen for extremes in osmotic stress tolerance without the use of sophisticated imaging technology, but only if screening growth experiments are carefully established, well-managed and appropriately analysed. Lastly, there was no significant relationship in genotype rankings between leaf Na^+ concentration and osmotic stress tolerance (r = 0.0643, n.s.), indicating the potential to target and pyramid both traits to further improve overall salt tolerance in Bangladeshi wheat.

150 diverse BMWRI wheat lines

The 150 wheat lines representing genetic diversity in the BARI (now BWMRI) wheat breeding program were selected by BWMRI wheat breeders and screened for two key salt tolerance traits:

(i) Leaf Na^+ exclusion and K^+ concentrations
(ii) Osmotic stress tolerance.

(i) leaf Na^+ exclusion and K^+ concentrations

A series of five large screening experiments were completed, where seedlings were grown in supported hydroponics at 150 mM NaCl over three weeks and harvested leaves were analysed for Na^+ and K^+ by ICP. There was 2.5-fold range in leaf Na^+ concentration amongst the wheat genotypes ranging from 132 – 309 μmol gDW^{-1}, with an average leaf Na^+ of 218 μmol gDW^{-1} (Figure 7.3.5b). This reasonably small range in Na^+ exclusion capability at low leaf Na^+ concentrations is not surprising from a collection of hexaploid wheats, where the *KNa1* gene for Na^+ exclusion is virtually ubiquitous. The small number of lines with the lowest leaf Na^+ concentrations, most likely contain additional minor genes or modifiers for Na^+ exclusion and would be worth targeting in a breeding program to further increase salt tolerance. There was less variation in K^+ concentration within the collection of wheat lines (1.5 fold) which was generally inversely proportional to Na^+ concentration (r = 0.538, P<0.05). Key lines with the lowest Na^+ concentration, generally had higher K^+ concentrations and the highest K:Na ratio.

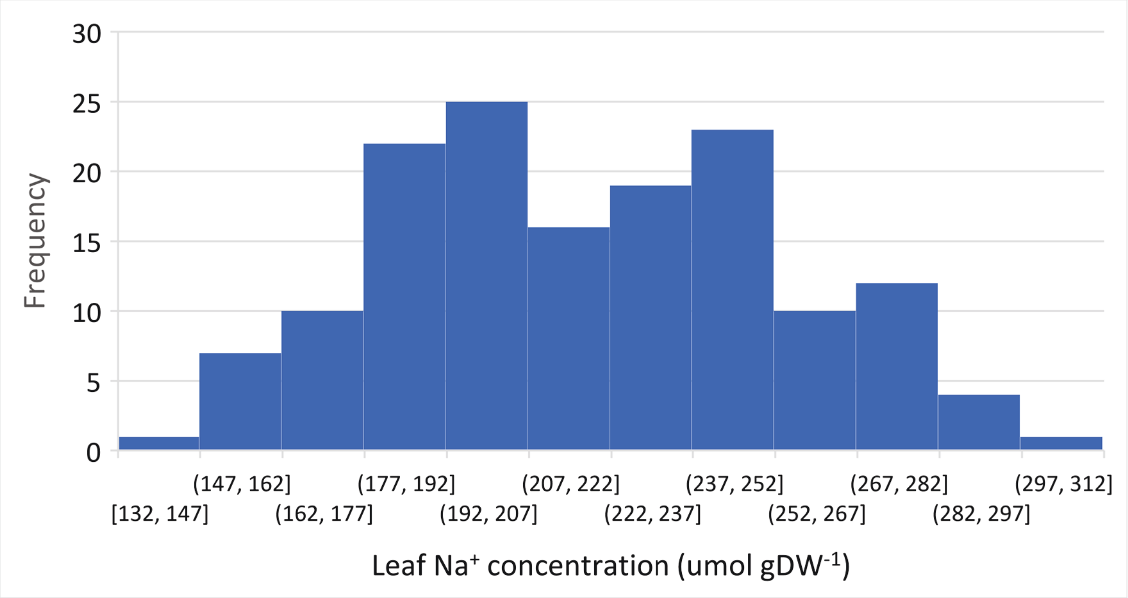

Figure 7.3.5b Frequency distribution of leaf Na^+ concentration after 10 d in 150 mM NaCl of 150 diverse BWMRI wheat lines. Bracketed numbers indicate the range in Na^+ concentration within each bin.

Prior to a targeted breeding program, an additional validation screen for leaf Na^+ concentration is recommended to confirm the low Na^+ status of lines suitable for use by BWMRI wheat breeders.

(ii) Osmotic stress tolerance.

Osmotic stress tolerance was assessed as the ratio of the RGR of wheat seedlings grown in a salt treatment versus control at a number of time intervals after the commencement of the salt treatment. The time interval with the most significant interaction between differential responses of lines to salinity levels was between 22 – 26 days after planting (DAP). The salt treatment at this time interval resulted in a significant reduction in RGR relative to controls - on average by 30%, which was less of a reduction than for experiments run at CSIRO for a similar salinity treatment (~45% reduction). There was a relatively small range in osmotic stress tolerance of 0.57 – 0.88 among the collection of 150 BWMRI wheat lines which followed a normal distribution (Figure 3.5c). A number of advanced breeding lines (BAW lines) were ranked as the most osmotic stress tolerant with RGRs due to salinity reduced only by between 12 – 20%. These lines include BAW 1299, BAW1045, BAW 1047, BAW 1329, BAW 1065. Repeat validation screens are recommended to confirm line rankings for this salt tolerance trait. Targeting osmotic stress tolerance in a breeding program is possible. As phenotyping this complex trait is not straightforward, comprehensive field testing on well characterised saline sites targeting yield of large populations derived from these lines crossed with best adapted parents will be required.

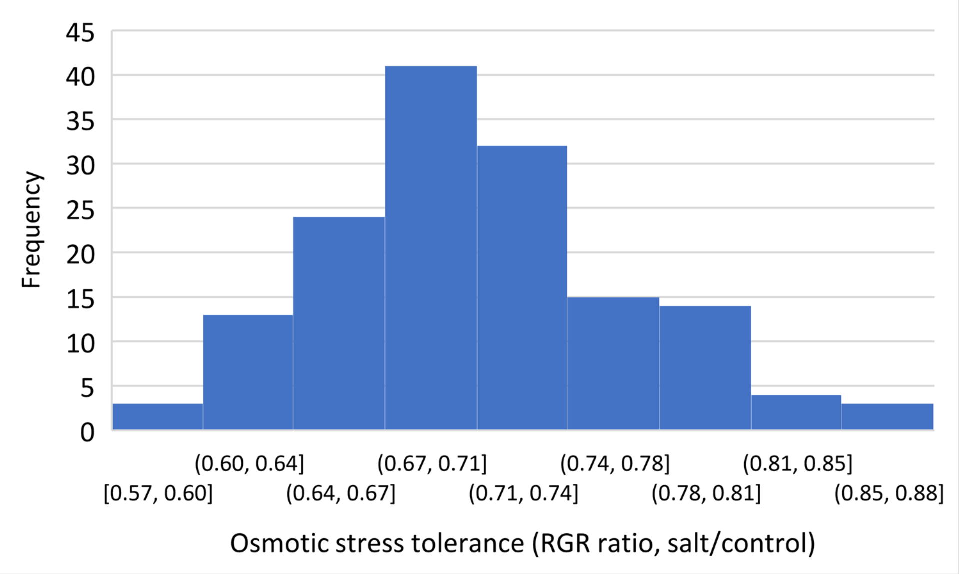

Figure 3.5c. Frequency distribution of the osmotic stress tolerance of 150 diverse BWMRI wheat lines. Bracketed numbers indicate the range in osmotic stress tolerance within each bin.

Objective 4: To identify germplasm of pulses and forages with tolerance to salinity and water-logging stress adapted to Southern Bangladesh.

The ARF report (7.1.1) emphasized the importance of waterlogging on pulse production, and as a result an increased project emphasis was made on WL tolerance screening of pulses under Objective 4. Early sowing coupled with WL tolerance was recognised as a way in pulses to avoid high salinity levels. Salinity build-up during the dry season was widely seen as another key issue for which tolerance was sought in pulses.

Waterlogging (WL) tolerance

Comparison among species for waterlogging tolerance: The yield of legume crops is reduced by soil waterlogging (WL) and tolerance to WL varies between and within grain legume species. Mr Edi Wiraguna (UWA PhD student - see 8.2 Capacity Impacts) evaluated the tolerance of four grain legume species - grasspea (3 genotypes), lentil (2), faba bean (2) and field pea (1) - to WL and anoxia (Wiraguna et al. 2021). There was variation between and within legume species in response to WL and anoxia. Grasspea was more tolerant to WL and anoxia than the other species confirming farmer perceptions.

WL tolerance in mungbean: Transient waterlogging (WL) in mungbean is a problem at different growth stages. At UWA to develop screening protocols for WL stress at germination and at the seedling stage a range of waterlogging duration treatments (0, 1, 2, 3, 4, 5, 6, 7, 8 days at germination & 0, 2, 4, 8, 16 days at seedling stage) was studied with mungbean (2) and blackgram (1) genotypes by PhD student Ms Khin Lay Kyu (John Allwright Fellow {JAF}) - see 8.2. (Khin Lay et al. 2021). We evaluated the responses to different durations of transient waterlogging in the glasshouse. Waterlogging reduced soil redox potential, delayed or even prevented germination, decreased seedling establishment and affected shoot and root development. Both species were delayed in emergence in response to short periods of transient waterlogging at germination, and with longer waterlogging duration germination and emergence failed, whereas at the seedling stage both crops failed to emerge. At the seedling stage the species showed adaptation by the formation of adventitious roots.

Building on the pilot study, systematic screening of germplasm for water logging tolerance was undertaken in the glasshouse and phenotypic variation in waterlogging tolerance was assessed in the mungbean mini-core collection at germination and at the seedling stage. At germination, the variation in % emergence under WL varied from 0-80% over accessions with tolerance related to the maintenance of seed viability under hypoxia coupled with emergence on the release of hypoxia. At the seedling stage, there were dramatic difference in waterlogging ability among germplasm lines with WL adaptation associated with adventitious root development. A high broad-sense heritability estimate among traits was found for seed emergence (H^2=81%) at the germination stage. The heritability for the formation of adventitious roots at the seedling stage in response to WL was H^2=56%. Genome-wide association studies (GWAS) was used to identify significant marker - trait associations with waterlogging tolerance. A total of 10,224 high-quality SNPs were used to identify significant loci associated with waterlogging tolerance and possible underlying candidate genes. At the germination stage, zinc finger protein (ZFP8) is a candidate gene for emergence after 4 days of waterlogging. At the seedling stage, FGGY carbohydrate kinase domain-containing protein was associated with adventitious root formation. The heritability estimates combined with the identification of sources of waterlogging tolerance demonstrate that it will be possible to select for these traits and accelerate mungbean breeding for climate resilient cultivars.

WL tolerance in grasspea

Grasspea seeds are exposed to soil waterlogging when relay sown under near-mature rice crops in south Asia. Relay sowing can cause seeds to experience hypoxia and/or anoxia due to soil waterlogging. At UWA following a protocol development experiment, we evaluated the waterlogging tolerance of a diversity panel of grasspea germplasm to identify sources of tolerance. A total of 53 diverse grasspea genotypes were waterlogged for 6 days and drained for 8 days. There was significant variation among grasspea genotypes in waterlogging tolerance. Three contrasting seedling response patterns were identified, and representative germplasm tested to identify the effect of seed priming (Wiraguna et al. 2020). Grasspea – a crop domesticated in the Near East – collected from Bangladesh and Ethiopia was more adapted to waterlogging at germination than material from other origins.

Mr Wiraguna studied the mechanisms involved in these contrasting responses to soil waterlogging. Critical oxygen pressure and respiration rate were not associated with the waterlogging tolerance of grasspea seeds. Waterlogging tolerant genotypes are more tolerant of hypoxia and anoxia than waterlogging sensitive genotypes. A low imbibition rate in the beginning of seed submergence of waterlogging tolerant genotypes is associated with hypoxia and anoxia tolerance (Wiraguna 2021).

At BARI, using protocols developed at UWA and transferred to BARI, the waterlogging tolerance of existing cultivars of grasspea was evaluated at both the germination and seedling stages in Yr3. At the germination stage five local cultivars were waterlogged for six days. Emergence varied from 28-49% among cultivars in comparison to 100% emergence in the drained control. In a separate experiment comparing the same cultivars at the seedling stage, the five cultivars responded similarly to waterlogging with differences among cultivars non-significant. In Yr4 local grasspea germplasm were evaluated at both the germination and seedling stages at BARI. Sources of waterlogging tolerance were identified.

WL tolerance in Field Pea

Field pea is often sown as a relay crop and subject to waterlogging stress in Bangladesh. At UWA as part of the PhD thesis of Md Shahin uz Zaman (JAF), we studied the response to WL stress at the germination stage in a recombinant inbred line (RIL) population (derived from a bi-parental cross between WL contrasting parents) and a diversity panel to identify extreme phenotypes, understand the genetic basis of WL tolerance and identify traits for possible use in indirect selection (Zaman et al., 2019). A wide range of variation in the ability to germination in waterlogged soil was observed in the RIL population (6 - 93%) and the diversity panel (5 - 100%) with a high broad-sense heritability (H^2 > 85%). The continuous distribution of the variation suggested polygenic control. In the diversity panel, a one-way ANOVA by region of origin showed that geographic region accounted for significant variation in WL tolerance at germination with genotypes from Africa (i.e. Ethiopia in study) showing the highest germination (80%) on average when exposed to waterlogged soil. Testa integrity, measured by electrical conductivity (EC) of the leakage solute, was strongly associated with WL tolerance in the RIL population ($r_G = -1.00$) and the diversity panel ($r_G = -0.90$). Therefore, testa integrity can be effectively used in indirect selection for waterlogging tolerance. Response to selection for WL tolerance at germination is confidently predicted, enabling the adaptation of the ancient model pea to extreme precipitation events at germination.

WL tolerance in lentil

The germination of lentil seeds can be affected by waterlogging caused by relay sowing into standing rice crops in South Asia. There is a consequent demand for waterlogging-adapted genotypes, particularly at germination. We investigated the effects of waterlogging at germination on a lentil germplasm collection; Firstly, with two WL-contrasting genotypes waterlogged for different durations (0, 3, 6, 9, 14 and 21 days) in pot soil system in the glasshouse; then on a diversity panel of genotypes (127) from 11 countries (Wiraguna et al. 2017). Finally, all 127 genotypes were phenotyped for morphological traits in pots. There was significant variation among genotypes, and those originating from Bangladesh had the highest germination under waterlogging at 21.2%. The heritability of germination on waterlogged soil among genotypes was intermediate at H^2 = 44.8%. It was concluded that Bangladeshi genotypes are adapted to waterlogging at germination. With an intermediate heritability for germination under waterlogging conditions, selection in lentil for relay sowing through waterlogging tolerance at germination is practicable.

Salinity tolerance

For salinity tolerance, protocols for pulse screening established at UWA were transferred to BARI at the 2018 Experimental Methods Training Workshop - as in wheat.

Meanwhile, at UWA Mr Shahin Iqbal (JAF) commenced his PhD study on salinity tolerance of mungbean in 2020 (See Section 8.2). His first experiment was to investigate the effect of salinity stress at the seedling and reproductive stages of mungbean. Four diverse mungbean genotypes, including BARI Mung-6, were grown in a glasshouse and five salinity treatments (0 mM NaCl (control), 25, 50, 75 and 125 mM NaCl) applied 15 days after sowing. Plants exposed to salinity stress developed foliar injury symptoms with the level of injury symptoms increasing with salinity stress. Genotypic differences were most prominent at maturity, particularly at 75 mM NaCl stress where the reduction rate of dry mass compared to non-saline control across over the salinity treatments was highest in BARI Mung-6 followed by VO1317, Jade AU and VO2211. Salinity stress severely reduced the numbers of flowers, pods and seeds per plant - ultimately reducing seed yield. Even at the lowest salt stress treatment (25 mM NaCl), cultivar BARI Mung 6 was affected. Mr Iqbal also studied the mechanisms involved in the contrasting responses to salinity stress. For example, Jade AU accumulated lower Na+ concentrations in young leaves compared to BARI Mung-6 in high salinity treatments. We also investigated the cellular and sub-cellular distribution of Na+, Cl- and K+ in frozen-hydrated young leaves of two genotypes (Jade AU and BARI Mung-6) grown in either 0 mM or 75 mM NaCl treatment using Cryo SEM X-ray microanalysis. Jade AU maintained higher K+/Na+ ratio in vacuoles and chloroplasts of mesophyll cells than BARI Mung-6 in saline conditions. Vacuolar Cl- concentration was similar in both genotypes, but Jade AU had lower Cl- in chloroplasts than BARI Mung-6 in salinity stress. Higher Cl- concentration in leaves caused leaf anatomical disarrangements, as a result, both stomatal and non-stomatal factors limited photosynthesis. Thus, leaf Na+ 'exclusion' and Cl- 'tissue tolerance' capacity appear to explain differences in salinity tolerance in mungbean.

Based on the first experiment, screening of the mungbean mini-core collection (292 accessions) under CIM-2014-079 for salinity tolerance at the vegetative stage were completed in 2021. Then, 130 diverse genotypes were selected from the vegetative stage screening for evaluation for salinity tolerance at the reproductive stage in 2022. Major phenotypic variation was observed for all 21 agronomic traits across three growth stages. The variation in reduction of shoot dry mass (% of control) varied from 48-90% at the seedling stage and from 45-86% at the vegetative stage. At the reproductive stage, the variation in seed yield reduction varied from 0-100% with a mean of 45%. Leaf injury under salinity stress and the reduction of shoot dry mass at both seedling and vegetative stages were significantly correlated with percentage of yield reduction. Based on Illumina resequencing of the 292 mini-core collection undertaken by WorldVeg Centre under CIM-2014-079, Mr Md Iqbal is undertaking GWAS analysis of the phenotyping data to understand the genetic basis of salinity tolerance in mungbean at seedling, vegetative and reproductive stages.

Activity 4.3 Field evaluation of germplasm of salt tolerant fodder options

The acute shortage of quality feeds and fodder is one of the most important obstacles to increased productivity in livestock in Southern Bangladesh. Our attempts to introduce into Bangladesh from Australia seed - each with their rhizobia - of cultivars of pasture legumes (Messina, balansa clover and burr medic) with salt and waterlogging tolerance for fodder production to BARI were unsuccessful. The major issue was Horticulture Exports inaction in Australia despite a complaint made by the project made against this Federal Department. The major stumbling block was the classification of *M. siculus* as a weed species in the MiCOR system of the federal Department of Agriculture.

In an alternative approach to improve the availability of fodder locally, a field study was conducted to evaluate existing pulse crops as forages in a farmer's field in Barisal in the *rabi* season 2020-21. Grasspea produced significantly more dry matter than field pea, but the converse was true for green forage yield indicating the higher water content of field pea. The highest gross margin (Tk138,690/ha) and BCR (7.93) were found with field pea - BARI Motor-3. Even grasspea returned an attractive BCR > 4, indicating the profitability of growing these pulses for forage. The experiment was re-sown the following season but destroyed by flooding.

New Activity 4.4 UWA completion of experimental work for: 1. Improved protocol for genetic modification procedures; 2. Standard operating practice (SOP) for clonal propagation of mungbean and 3. SOP for year-round accelerated Single Seed Descent (aSSD)

Despite genetic transformation emerging as a revolution in crop improvement, for many crops, including legumes, efficient transformation and complete plant regeneration remain a challenge. There is an urgent need to optimise protocols for the delivery of CRISPR or conventional genetic transformation. In this project, we explored factors affecting Agrobacterium-plant tissue interactions to increase genetic modification efficiency. The effect of light has been studied in plants and bacteria. Agrobacterium grows better in the dark as light reduces flagella mobility. In plants, light quality (spectra) and duration influence plant development. However, there is little published on *in vitro* culture. We aimed to provide an easy, cost-effective, and reliable genetic modification protocol to boost fundamental studies on gene function and facilitate crop improvement.

In terms of regeneration, we observed direct organogenesis, i.e., no undifferentiated callus formation was observed. Shoot regeneration media (SRM) supplemented with activated charcoal (AC) showed 100% of shoot regeneration, whereas without AC was 80%. For further experiments, SRM supplemented with 2.5 g/L AC was selected, as some degree of hyperhydration was observed on explants without AC (Fig. 3c and D).

Among the different concentrations of Hyg tested to select transformed shoots, we choose 25mg/l. In addition, 300mg/ L of cefotaxime did not show any negative effect on shoot regeneration. Therefore, shoot media were supplemented with Hyg 25mg/L and cefotaxime 300 mg/L.

After these parameters were established, we tested the transformation efficiency under three different light spectra. Under blue and red enriched protocols, non-transformed controls showed a lower proportion of explant showing regeneration (0.64 under red and 0.39 under blue) compared with the ones regenerated under fluorescent lights (0.83). Those that went through transformation (Agrobacterium co-culture) showed a 20% decrease in the proportion of explants showing regeneration across lights. As expected, we found differences in the proportion of explant showing at last one GFP positive shoot: There was no difference between explants which went through co-cultivation under fluorescence or red-enriched lights (0.58 and 0.44, respectively) but under blue-enriched light proportion was lower (0.19). After shoot regeneration, we observed a detrimental effect of cefotaxime on shoot development and rooting. This effect needs to be study and there is a need to find an antibiotic alternative to cefotaxime in this system.

Clonal propagation: Within the context of a breeding program, a clonal propagation protocol is useful to multiply seeds when a specific plant is identified presenting with a specific trait of interest - such as salinity or waterlogging tolerance or to increase - for example - the number of F_2 seeds from a cross consisting of a single F_1 plant. We found vegetative growth was unaffected by photoperiod. We established the pre-flowering stage at 4 - 5 weeks (28 to 35 days) after sowing, as the optimal period for clonal propagation. The hormones IAA, IBA and NAA induced adventitious root formation in all cultivars, except for Onyx-AU that did not form roots with IAA. The greatest rooting (up to 80 %) was observed with the IBA gel (commercial formulation), independently of the species and cultivar. Therefore, we recommend using the IBA hormone gel when possible.

accelerated Single Seed Descent (aSSD): In traditional agriculture single seed descent (SSD) protocols are limited by the plant environmental requirements, i.e., it can be only performed in-season, in the worst case once a year. In legumes, such as pea, chickpea, and soybean, there are known protocols for accelerated SSD (aSSD). These protocols are based on artificial environments using optimised *in vivo* growth parameters for a given species, including light, photoperiod and temperature, to induce early floral onset and seed maturation to reduce generation time (Croser et al. 2016, Ribalta et al. 2017). Thereby, we can create a season- (year-round) and genotype-independent system.

Plants from the different cultivars representing *V. radiata* and *V. mungo* grew and developed normally under the three environmental conditions with all plants developing viable seeds. Among the three environments tested, in ENV1 (in-season) the number of days to first flower and generation time (days from sowing to harvest of a seed to continue to the next generation) were faster or equal to ENV2 (13h-artificial). However, the fact that time to harvest is similar or slightly faster in ENV1 versus 2 means that we can use ENV2 as optimal for year-round SSD. A generation time average of 60 days (up to 6 generations a year) has also been reported in other legumes such as field pea, chickpea or lentils (Croser et al. 2016, Ribalta et al 2017) with a similar approach. In our case, no harvesting of immature seeds and subsequent treatment was involved making this protocol simple.

8 Impacts

8.1 Scientific impacts – now and in 5 years

During the project Australian scientists from UWA and CSIRO worked closely with national scientists to jointly develop the project experimental program annually during in the reporting/planning workshops (and soon thereafter). Research implementation in Bangladesh was predominantly a national scientist activity, while writing-up research results was a joint affair. Reports of each component activity were first-drafted by national scientists and then the summaries were reported in the Annual Research Reports and, herein, in the Final Report. We also jointly wrote international scientific publications. Such publications will attract a wider scientific community reflecting both scientific and capability impacts. Unfortunately, due to local and international travel restrictions from COVID, shoulder-to-shoulder interactions of international with national scientists and national to regionally-based scientists were restricted.

Publications from the project are listed in Appendix 1 and include 12 international journal articles, 6 conference paper/posters, and 4 PhD and 1 MSc theses completed with 2 theses on-going. As an example, from the PhD research of Md Zaman (see 8.2) we published an article of Zaman et al. (2019) entitled *Changes in gene expression during germination reveal pea genotypes with either “quiescence” or “escape” mechanisms of waterlogging tolerance* in *Plant, Cell & Environment* which has the high impact factor of 7.947 and received 23 citations to-date.

Publications are easily quantifiable, but less easy to measure is the improved performance and scientific impact from the methodologies transferred and the working shoulder-to-shoulder in planning and writing up the research. This leads into the next section (8.2) - Capability Impacts.

In wheat, as an example (parallel activities were undertake for pulses), a key impact of the project has been the development and adoption of infrastructure and methodologies to screen for salt tolerance traits. This was enabled by the procurement of supported hydroponics infrastructure and the development a comprehensive methods manual, together with running an Experimental Methods Training Workshop at BARI in Gazipur. Another set of key impacts of this project has been the establishment and adoption of tailored experimental field trial designs, detailed soil characterisation principles and practices for running field trials in salt-affected soils and appropriate statistical analysis protocols including the implementation of covariates in the analysis of yield. These principles and practises were outlined in the Experimental Methods Training Workshop and subsequently adopted in the establishment and completion of numerous field trials over five seasons in southern Bangladesh by the BWMRI team.

This knowledge and capability will be important for ongoing use over the next 3 to 5 years, by BWMRI wheat breeders and BARI physiologists to not only validate the most promising lines from the 150 diverse wheats screened for salt tolerance traits during the project, but to also provide ongoing screening capacity to phenotypically select for lines in a breeding program. This will be particularly relevant in selection for osmotic stress tolerance which is genetically complex and currently devoid of molecular tools to aid selection. It is likely that minor genes for Na^+ exclusion can be identified in the 150 diverse wheats of the BWMRI wheat breeding program and either pyramided with osmotic stress tolerance or added to the *Nax* breeding program (see below) to generate greater salt tolerance.

A project publication produced which outlined a novel way to assign salt tolerance rankings in the field based on the slope of yield decline in response to salinity (underpinned by relevant design and characterisation components) has the potential to radically change the way scientists and breeders evaluate and rank wheat salt tolerance in the field and lead to a more accurate method to select wheats that are actually salt tolerant and not just well adapted to the target environment in terms of yield potential.

In the pulses we can anticipate scientific impact from opportunistic linkages firstly forged with Project CIM-2014-079 *Establishing the international mungbean improvement network* and with the World Vegetable Centre on mungbean, and secondly with on-going UWA research on genetic transformation and rapid generation systems in cool-season pulses. Using the WorldVeg mini-core germplasm collection of mungbean and their associated sequence data, we were able to identify not only waterlogging tolerant mungbean accessions but also candidate genes for WL tolerance. We anticipate a similar output for mungbean salinity tolerance. The whole area of waterlogging tolerance in pulses was prior to this project very under-studied, the project's student research on WL in grasspea, lentil mungbean, and pea is all novel. Leveraging previous research on cool-season legumes funded by Grains Research and Development Corporation (GRDC) to the project leader, we were able to develop some generic research methods/tools for mungbean - a warm-season pulse - at low cost by capitalising on existing skills and staffing at UWA. Such outputs included an improved protocol for genetic modification, a methodology for clonal propagation, and a system for year-round accelerated Single Seed Descent (aSSD). Generic research methods/tools have a high probability of future scientific impact.

8.2 Capacity impacts – now and in 5 years

Within the project as capacity impacts we can highlight post-graduate training undertaken through research on project-related themes. At UWA project-associated PhD students were:

- Md Shahin Uz Zaman (Bangladesh) - PhD (John Allwright Fellow [JAF]) on *Waterlogging tolerance at germination in pea* - Completed 2019 and returned to BARI, Bangladesh
- Edi Wiraguna (Indonesia) – PhD on *Waterlogging tolerance in grasspea* – Completed 2021 and returned to Indonesia
- Khin Lay Kyu (Myanmar) – PhD (JAF) on *Waterlogging tolerance in mungbean* - started 2018 and submitted
- Md Shahin Iqbal (Bangladesh) - PhD (JAF) on *Salinity tolerance in mungbean* - started 2020 and on-going

In Bangladesh at BAU, Professor Taj Uddin conducted the baseline socio-economic survey in 2018/19 with Honours and Masters students, who received on-the-job training in socio-economic survey methodology. Data collected were used for the Masters thesis of Md. Mominul Islam entitled: *Farming Practices and Livelihood Status of Non-saline and Saline Farm Households in Southern Bangladesh: A Socioeconomic Study*, submitted in Semester 2, 2019.

A BARI scientist - Mr. Nanda Dulal Kundu (Scientific Officer, Madaripur) - enrolled in 2021 for his PhD under the supervision of Professor Uddin in the BAU Economics Department. His thesis topic is *Climatic Stresses, Adaptation Strategies and Capacity Assessment of Pulses Growing Farmers in the Coastal Areas of Bangladesh*. He is sponsored by the project.

The above post-graduate capacity building from the project may be anticipated to have sizeable future impact. The project covered operational funding for student research while their other costs were variously co-funded.

Dr. M. G. Neogi, Deputy Project Leader, University of Western Australia received the highest state award "Independence Award-2021" from the Bangladesh government for his outstanding lifetime contribution to science and technology. The honourable Prime

Minister of Bangladesh Sheikh Hasina presented this prestigious state award at Ganabhaban (Prime Minister's Office), Dhaka on 20 May 2021. Dr. Neogi worked with UWA as Deputy Project Leader from 2017. Previously he led research/development on *Monga* (famine from September to early November) mitigation in northern Bangladesh for ~18 years. He developed a short duration rice-based cropping pattern, with which farm households were able to harvest their monsoon rice (*aman*) in September/October instead of late November allowing the timely planting of the succeeding crop to maximize yield and overcome food insecurity. The award afforded Dr Neogi access to political and government officials to the highest level in Bangladesh which facilitated project activities and with links into government departments for follow-up.

8.3 Community impacts – now and in 5 years

8.3.1 Economic impacts

Positive economic returns from pulse cultivation: The extensive program of field activities - particularly demonstrations of dry-season pulses - under CIM-2014-076 project showed benefit-cost ratios above 2.0 for production packages on mungbean, relay pea, cowpea, relay grasspea and lentil indicating their profitability. Considering mungbean specifically, the tested demonstration package comprised line sowing (30 cm apart) in the month of January at 25 kg seed/ha of cultivar (BARI Mung 6) with fertilizer (NPKS 20-40-20-10 kg/ha). Insecticide was applied (Volume Flexi @ 0.5ml/l two times [before and 15 days after flowering]) to control thrips and pod borer. In 40 demonstrations conducted over the project duration, the average yield gap between demonstration package and adjacent farmer plots was 289 kg/ha indicating a yield advantage of 29%. For example, mung bean farmers are getting higher economic benefit due to early planting i.e. in January which enables them to harvest more mungbean and at least one extra harvest/pick and also avoid drought and possible salinity build-up issues on the crop.

Feedback from grower households was solicited at field days and during farm visits. Farmers confirmed that mungbean mechanical planting by seeder helps reduce their production costs as well as ensure increased production. However, the lack of availability of suitable seeders in the community is a major policy issue for efficient mungbean cultivation that remains to be addressed.

Some pulse demonstrations were lost to the most common reason - flooding. Temporary inundation is unpredictable. It is clear in the field: *No possibility of drainage; No mungbean* is a key mantra. Waterlogging tolerance in mungbean was a major theme in the project's back-up research, and the incorporation of identified sources of tolerance into national varietal development provides a way forward.

A scaling-up issue raised by mungbean growers is cattle grazing. After the harvest of *aman* rice, cattle movement for grazing is difficult to combine with mung cultivation in dry season cropping. Large cropped blocks are required to manage cattle grazing and for this coordination with local Union Parishad Chairman and Members is needed.

Positive future for cowpea in Barisal Division: Cowpea is a traditional crop of the coastal region that is usually cultivated by relaying into *aman* rice with sowing methodology and timing like grasspea. Results from last three years piloting programs shows that average yield of cowpea ranges from 1.24-1.50 t/ha in the region. In Yr4 the benefit-cost ratio for cowpea production ranged from 2.1 in Bhola to a very encouraging 3.27 in Patuakhali. The yield gap and the cost-benefit ratio over the years particularly in Patuakhali should encourage cowpea uptake as a dry-season cropping option. Furthermore, we will be pushing the release and seed multiplication of the very promising introduced cowpea line CPL-8-17 to further boost cowpea in Southern Bangladesh in 2023 and another early high yielding line in the following year.

Development of new salt tolerant bread wheat varieties: This project established clear proof of concept that salt tolerance *Nax* genes in modern Bangladeshi bread wheat varieties are effective in reducing leaf Na^+ concentrations and consequently improve yields in moderate to high saline soils in southern Bangladesh. What will be required next is a targeted breeding program by the BWMRI wheat breeders where the most promising *Nax1* and *Nax2* lines in the BARI Gom 25 background are crossed into a range of the best locally-adapted advanced breeding lines in terms of yield, quality and disease resistance – particularly resistance to wheat blast. The development and ongoing availability of robust and informative molecular markers optimised for use in Bangladeshi wheat germplasm, and the capacity to validate the phenotype through controlled environment screening, will aid in the efficient selection of target progeny in a breeding program, leading to the eventual release of new salt tolerant bread wheat varieties, suitable and adapted to be grown in southern Bangladesh. These new varieties are likely to yield up to 20% higher on challenging saline soils than currently used varieties.

A project publication produced which provided thorough scientific validation of effectiveness of the *Nax* genes to improve salt tolerance and subsequent yields of bread wheat grown on saline soils in southern Bangladesh, will inevitably speed the uptake and adoption of bread wheats containing the *Nax* genes into wheat breeding programs in south and central Asia (e.g. India, Pakistan) and adoption more broadly in countries with significant salinity issues both in irrigated and dryland agriculture.

8.3.2 Social impacts

Mungbean mini mills: To-date the project has established 21 mungbean de-husking mini mills in the project area, with ten more in the process of establishment in remote mungbean production areas. Introducing mungbean mini mills is impacting coastal farmers and households in Bangladesh. Mungbean is the most important dry-season pulse crop in the region. The project has demonstrated ways to increase its production and profitability (see above). Despite this large production, due to the absence of mungbean de-husking mini mill facilities in, for example, Barguna district, farmers used to sell their unprocessed mungbean at Tk 50-60 per kg. On an average, only 10% of households consume high-protein mungbean grain by using *Shill*, *Pata* and other local and traditional de-husking systems, which are highly laborious and time-consuming processes for women. De-husking facilities are widely available for rice and wheat grain; but farmers were unable to de-husk mungbean due to the local absence of de-husking equipment. In this situation, a mini mill was established by PEP in the project at west Kewrabunia village of Barguna district and given to Mr. Abul Kalam Sentu. Gradually the number of mini mills was increased from one (1) to 21 in different locations of the project. (More are being installed). Using these mini mills farmers are able to de-husk mungbean at Tk 10 per kg and then sell it in the market at Tk 120-140 per kg. Cost Benefit Analysis shows that the margin for per kg mungbean was Tk 70-80. Anecdotal evidence suggests that farmers are now able to sell mungbean at an increased price impacting their income and that with the use of these mini mills to de-husk mungbean, there is now a knock-on effect of improved household nutrition. Farm households appear to now benefit both economically and nutritionally. Currently farmers are happy with the establishment of mini mills in remote areas. “We are happy for getting mini mills from PEP and confident to grow more mungbean in future for economic resilience. The tendency of consuming mungbean by our families will increase as we can have nutrition intake from it.” – Said several smiling farmers. Mr. Zobaidul Alam, Deputy Director of DAE of Barguna district inspected the mini mills recently. He mentioned that these mini mills will contribute to poverty reduction of coastal communities. He also suggested to establish mini mills in every Upazilla of the district for more benefits. Mini mill receiver Abul Kalam (Sentu) expressed with joy “I got an employment opportunity as Poverty Eradication Program (PEP) gave me a mini mill free of cost and my family is running well with the income from it. I would like to extend my gratitude to the organization”. The Bangladesh Minister for Agriculture and Secretary for Agriculture after visiting with the project now wishes to extend our pilot mini mills concept with approximately one hundred more mini mills for Patuakhali and Barguna districts. The increased return to households from local mini mill de-husking of mungbean is now catalysing greater local interest in the crop mungbean. The current general under-investment in fertilizer and crop protection for optimum return is being re-considered and there is anecdotal evidence that the availability mini mills in the community galvanises greater attention to in-field crop management, including increased use of inputs, to maximise return and crop production.

Relay green pea for women in the household: Farmers are very happy to see the outcomes of relay green pea in aman (monsoon) rice crops which was introduced by the project in the last two years. Pea growing households indicate that it is a new crop in the region with gender implications. Field pea for green pods taken as a relay crop is considered as a low input cost technology and is easily handled by women-headed households. Green pods of pea can be harvested weekly by women in the household, and then sold in the local market with very good demand at c. Tk 40-50/kg, so the technology is encouraging for households to adopt. With no apparent pest/disease infestation during the crop life cycle, hence no requirement for agrochemicals, green peas can safely be consumed raw without processing. This additional crop appears to help improve children's as well as the rest of the family's nutrition as most children like it as green pea. After the harvest of field pea, mungbean can be cultivated on the same land, giving scope for further cropping intensification. The crop has particular promise as a green vegetable, rather than as a grain, because of the recent completion of the Padma Multipurpose bridge which opens major new export markets in Dhaka (See Section 2.2.5).

8.3.3 Environmental impacts

The inclusion of pulse crops typically benefits succeeding crops by improving soil health as a result of biological nitrogen fixation and other rotational effects. Mungbean, cowpea, grasspea, lentil and pea - all fix atmospheric nitrogen in association with their Rhizobia. We anticipate knock-on improvements in soil health from an increase in future pulse cultivation.

8.4 Communication and dissemination activities

Regarding communication, a major focus was the demonstrations for both farmer training and field days, and we changed upazilla each season to widen the household catchment. Farmer training was in groups before sowing and later in the growing season (Table 8.4.1). It was usually crop-specific and given by PRC scientists with OFRD and PEP staff. We planned with OFRD to have many training sessions in the 2021 harvest season in the no-cost extension period of KGF funding. The figures for 2021 reflect pre-sowing sessions before the introduction of COVID restrictions, to which we adhered closely. Overall, there were a total of 45 groups of farmers totalling 1,453 farmers trained, of whom 323 or 22.2% were female.

Field days exhibited the demonstrations of crops to neighbouring households just before harvest. As with training, the field days were organised by OFRD - recently PEP - to include PRC researchers, NGOs, agriculture extension (DAE) field level workers and farmers to implement and achieve project objectives. The COVID restrictions varied over time, and we worked within the windows of possibility and safety. Over the five years we held a total of 28 field days and five mini mill demonstrations (ARF - 3 & PEP - 2). Overall, we had a total of 3,563 farmers in attendance at such field days/ mini mill demonstrations, of whom 780 or 22.8% were female (Table 8.4.2). Considering both training and field days we probably interacted with members of more than 5,000 households during the project.

Table 8.4.1 Farmer training by harvest year showing gender and number of batches.

Harvest year	No. of batches	No. of male farmers	No. of female farmers	Total no. farmers

2018	8	220	50	270
2019	10	270	103	373
2020	11	256	74	330
2021	16	384	96	480
Total	45	1,130	323	1,453

Table 8.4.2 Field Day attendance by harvest season, including mini mill demonstrations

Harvest year	No. of field days	No. of male farmers	No. of female farmers	Total no. farmers
2018	9	849	295	1,144
2019	6	384	96	480
2020	12	1,046	334	1,380
2021	1	200	24	224
	3*	N/A	N/A	150
2022	2**	154	31	185
Total	33	2,633	780	3,563

* Mini mill demonstrations by ARF; ** mini mill demo. by PEP.

Dr Neogi excelled in providing extensive media coverage in national newspapers, both English and Bengali, and also on television exposure throughout the project (Table 8.4.3).

In 2021 ARF published a booklet in *Bangla* on mungbean production, processing, marketing, nutrition, and value chain and distributed among the stakeholders and participating farmers. In 2022 PEP developed and printed 4,000 leaflets - in *Bangla* and English - on grass pea, on green pea and on mungbean production technologies and also 2,500 leaflets to introduce mungbean mini mills at the community level.

Table 8.4.3 Listing of media communications from the project

Date	Media	Subject/Title
11 July 2017	Broadcast in news on *Channel I and GTV*	The project's inception workshop held at BARI, Gazipur on July 11/12, 2017 reported.
24 Oct 2017	National English newspaper *Daily Sun*	*Climate-Smart Agriculture in Coastal Bangladesh* in relation to the project written by Dr. M. G. Neogi in which the cultivation of pulses in dry season in fallow coastal area was accorded high priority.
5/6 Mar 2019	Broadcast by *Bangla TV and My TV* in news	News on project travelling workshop
9 Mar 2019	*Daily Sun* newspaper	News on travelling workshop with photos as *Australian scientists visit wheat fields in Khulna*.
26 Mar 2019	Broadcast by *Channel I* TV	Wheat development program in salty soil at Dacope.
31 Mar 2019	*Daily Sun* newspaper	News on wheat development in salty soil such as Dacope and Koyra, as well as our Field Day on wheat at Dacope
11/12 Apr 2019	Video developed & broadcast by Bangla Vision TV	*Wheat production and development in salty soil - Dacope*.
3 May 2019	Video developed & broadcast by GTV	*Mungbean production, development and consumption*
26 June 2019	*Daily Sun* newspaper	*Cowpea – a stress tolerant most nutritious pulses crops*.
1 July 2019	*Kaler Kantha* National Bengali newspaper	*Mungbean cultivation in coastal region.*
31 July 2019	*Daily Sun* newspaper	*Grasspea and mungbean in coastal agriculture.* The article was about problems and opportunities to cultivate grasspea and mungbean.
3 Sept 2019	*Daily Sun* newspaper	Project mid-term review and annual review and planning workshop held from 1 -3 Sept. 2019 at BARI, Joydebpur.
4 Sept 2019	TV Broadcast: *Channel i, ATN Bangla and Baishakhi*	News about the meeting with the agriculture minister and Australian delegates.
5 Sept 2019	*Daily Sun* newspaper	News of meeting on 4th September 2019 of Agriculture Minister with Australian project delegates.
19 Nov 2019	National newspaper *The Financial Express*	*Australia-Bangladesh partnership in agricultural research*. The article featured the contribution of ACIAR to Bangladesh agricultural research over decades and future partnership opportunities.
6 Jan 2020	*Daily Sun* newspaper	*Salinity and stress-resistance crops for coastal Bangladesh.* The article was on the research outcomes of the project.
12 Mar 2020	Broadcast by c. 20 twenty TV channels	The visits of Dr. Md. Abdur Razzaque to research project site at Dacope and his interviews in national news.
Apr 2020	Published by Ministry of Information in *Bangladesh Quarterly* booklet	*Wheat can flourish in fallow land of coastal Bangladesh*. The article was on project outcomes on wheat research in the coastal region including meeting with Agriculture Minister Dr. Md. Abdur Razzaque on 4th September 2019.
8 Apr 2020	National newspaper *Bangladesh Pratidin*	*Crops cultivation in fallow land of coastal region*. The article was on the outcomes of project research.
May 2020	Published in *Bangladesh Quarterly*	*Cowpea – a stress tolerant most nutritious pulse crop* - This popular article was on project research outputs on cowpea.
July 2020	Published in *Bangladesh Quarterly*	*Greening the fallow land of coastal Bangladesh.* The article was about Australia-Bangladesh collaborative agric. research boosting cultivating fallow land in dry season and the visit of Agriculture Minister Dr. Razzaque to Dacope, Khulna on 12th March 2020.
13 July 2020	National newspaper *Samakal*	Article in Bengali *Mungbean cultivation in fallow land of coastal region.* The article - developed with ARF - emphasized opportunities for mungbean cultivation in southern region following project research.
21 Mar 2021	Broadcast TV *Channel I*	Video news on *Salt tolerant wheat is growing in saline soils* on Field Day at Hajipur, Kalapara, Patuakhali on 19 Mar. 2021
23 Mar 2021	*Kaler Kantha* newspaper	Australia-Bangladesh Collaboration Strategy 2021-2030
Mar 2021	ARF published a booklet in Bangla	Mungbean production, processing, nutrition, and value chain for distribution among the trainees, stakeholder and farmers.
23 Aug 2022	*Daily Sun* newspaper	Mini mills for mungbean in coastal region
27 Nov 2022	Channel i TV channel	Mungbean mini mills as outcomes of Australia-Bangladesh joint project 30 minutes story of mini mills telecast and as national news
26 Nov 2022	*Sangbad* Bengali national newspaper	Mungbean Mini Mill: A blessing to the coastal mungbean farm families
20 Dec 2022	*Amader Shomoy* Bengali national newspaper	Farmers are getting profit through mungbean mini mill services, written by Shykh Seraj
2 Jan 2022	*Daily Star*	Availability of milling machines changing farmers fortunes; Mungbean cultivation expanding in Patuakhali, written by Sohrab Hoassain

9 Conclusions and recommendations

In conclusion, for saline lands in Southern Bangladesh, the project identified technology options offering improved productivity and profitability to farm households were found by the project for a range of pulse crops: mungbean, cowpea, grasspea, green pea and lentil. For saline lands, abiotic stress tolerances were found for salinity in wheat and for waterlogging in pea, grasspea and mungbean that now need to be introgressed into locally adapted cultivars to realize the potential gains in productivity and profitability. As such the project was successful in its research, but its outputs now require follow-up to translate into changes at the poor household level in the central coastal region of Bangladesh as follows:

Project outputs applicable today:

- Extension of the production packages for saline-free land (incorporating variety, fertiliser, sowing method and rate, and plant protection measures) for mungbean, cowpea, grasspea, relay green pea, and relay lentil.
- Mungbean mini mill installation.
- Newly released salt tolerant wheat varieties BWMRI Gom 2 and BWMRI Gom 4.

Outputs applicable within one year:

- Cowpea varieties to be released in 2023
- Salinity tolerant grasspea varieties for release in 2023
- Action to ensure availability of seed of new varieties at the farm level
- Definition of recommendation domains for the above pulse production packages considering adaptation to climate change (i.e. relay lentil specifically on charland in Barisal District, and relay green pea on well-drained soil etc)

Achievable outputs that require sustained and concerted support to translate into farmer-ready innovations:

- Salinity tolerant varieties of wheat - using both the Nax genes and BAW1147 and incorporating earliness, heat tolerance and disease resistance - and of mungbean incorporating both the new salinity and waterlogging tolerances
- Evaluation of relay sowing of wheat in the coastal area
- Development of mobile (farm-gate) mini mill system

Longer-term outputs:

- Evaluation and incorporation of early vigour trait (with high biomass) into local wheats
- Mungbeans with synchronous maturity

This pathway describes the route to the translation of project results into improved smallholder welfare in the coastal region of Bangladesh.

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